CRANFIELD UNIVERSITY

Rui Plácido

Estimating Measurement Uncertainty In the Medical Laboratory

Cranfield Biotechnology Centre
Biomedical Laboratory Sciences

PhD
Academic Year: 2015 - 2016

Supervisors:
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Dr. Maria Adelina Gomes

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ABSTRACT

Medical Laboratories Accreditation is covered by ISO 15189:2012 - Medical Laboratories — R*equirements for Quality and Competence*. In Portugal, accreditation processes are held under the auspices of the Portuguese Accreditation Institute (IPAC), which applies the Portuguese edition (NP EN ISO 15189:2014). Accordingly, Medical Laboratories accreditation processes now require the estimate of measurement uncertainty (MU) associated to the results.

The Guide to the Expression of Uncertainty in Measurement (GUM) describes the calculation of MU, not contemplating the specific aspects of medical laboratory testing. Several models have been advocated, yet without a final consensus.

Given the lack of studies on MU in Portugal, especially on its application in the medical laboratory, it is the objective of this thesis to reach to a model that fulfils the IPAC's accreditation regulations, in regards to this specific requirement. The study was based on the implementation of two formulae (MU-A and MU-B), using the Quality Management System (QMS) data of an ISO 15189 Accredited Laboratory. Including the laboratory's two Cobas® 6000–c501 (Roche®) analysers (C_1 and C_2) the work focused three analytes: creatinine, glucose and total cholesterol.

The MU-B model formula, combining the standard uncertainties of the method's imprecision, of the calibrator's assigned value and from the pre-analytical variation, was considered the one best fitting to the laboratory's objectives and to the study's purposes, representing well the dispersion of values reasonably attributable to the measurand final result. Expanded Uncertainties were: *Creatinine* - C_1 = 9,60%; C_2 = 5,80%; *Glucose* - C_1 = 8,32%; C_2 = 8,34%; *Cholesterol* - C_1 = 4,00%; C_2 = 3,54 %.

This evidence was confirmed by IPAC's accreditation auditing (2015/2016), having the MU estimate presented contributed for the approval on the accreditation report and for the renewal of this recognition and certificate.

Nonetheless, in global terms, it stands as necessary the investment from Manufacturers, Reference Laboratories and International Scientific Organisations providing reference methods and certified reference material, with high metrological traceability, focusing on the calibrators, as well as on the internal quality control materials. General laboratory investment is also needed to improve practice in the pre-analytical phase, assessing and estimating each own specific pre-analytical uncertainty. In addition, implementing MU estimate in the medical laboratory will require explicit and consensual guidelines, new tables with goals and allowable limits for MU and education, regarding this new tool, targeting the technical and medical laboratory's staff, but also the laboratory users, physicians and patients.

Keywords:

Accreditation; ISO15189 Standard; Technical Requirement; Top-Down; Calibration Hierarchy; Traceability; Trueness; Bias; Intermediate Precision; Imprecision; Pre-analytical Phase; Variation

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And lastly, this is for my sons, Rodrigo e Duarte, who are the best of me and to whom I hope one day to be an example. I hope to be able to guide and inspire you; to lead you to be bold and wild and free; to teach you to dream and to believe, to want, to pursue and to conquer; always knowing you must to get up after you fall and that you should reach out to who you see falling; … in the end, I hope you to live boundless, full and happy.

Thank you all...

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- R. Plácido Raposo, A. Freitas, M. Gomes, S. Morgan, M. Faísca. (2013). Measurement Uncertainty in the Medical Laboratory - Implementation and Evaluation of Two Different Formulas in Clinical Chemistry Parameters: Total Cholesterol, Creatinine and Glucose Measurements. IFCC – EuroMedLab Milano 2013 – 19-23 May; Milano. 20th IFCC – EFLM European Congress of Clinical Chemistry and Laboratory Medicine. 45th Congress of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC).

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- R.M.P. Plácido Raposo. "Metrological Traceability and uncertainty associated with the results in the Medical Laboratory: Reality or Utopia?!" Oral Presentation in the IV Health Meeting: "New Horizons in Health", of the School of Health Sciences, University of Algarve, in October 2013.

LIST OF ABBREVIATIONS

Abbreviations for associations, committees, organisations and laboratories

AACC	American Association for Clinical Chemistry
BIPM	International Bureau of Weights and Measures
CITAC	Cooperation on International Traceability in Analytical Chemistry
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical Laboratory Standards Institute
CV %	Coefficient of Variation Values
CVPre %	Pre-Analytical Variation
EA	European co-operation for Accreditation
EC4	European Communities Confederation of Clinical Chemistry and Laboratory Medicine (*under the direction of EFLM*)
ECL	Electrochemiluminiscence Technology
EDMA	European Diagnostic Manufacturers Association
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine (*EFLM represents IFCC in Europe*)
EFLM WG-H	Working Group on Harmonisation of total testing process
EQAS	External Quality Assessment Scheme
EURACHEM	European Association for Analytical Chemistry
EUROLAB	European Federation of National Associations of Measurement, Testing and Analytical Laboratories

GUM	Guide to the Expression of Uncertainty in Measurement
IAF	International Accreditation Forum
ICHCLR	International Consortium for Harmonization of Clinical Laboratory Results
IDMS	Isotope Dilution Mass Spectrometry
IEC	International Electrotechnical Commission
IFCC	International Federation of Clinical Chemistry
ILAC	International Laboratory Accreditation Cooperation
INSA	National Health Institute – Dr. Ricardo Jorge
IPAC	Portuguese Institute for Accreditation
IPQ	Portuguese Quality Institute
IQC	Internal Quality Control
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IUPAP	International Union of Pure and Applied Physics
IVD	*In Vitro* Diagnostic (medical) Device
JCGM	Joint Committee for Guides in Metrology
JCTLM	Joint Committee for Traceability in Laboratory Medicine
JIFCC	Journal of the International Federation of Clinical Chemistry and Laboratory Medicine
MU	Measurement Uncertainty
NAB	National Accreditation Bodies

NC	Non-Conformity
GN	*Gnóstica - Medical Laboratory*
NPAAC	National Pathology Accreditation Advisory Council
OIML	International Organization of Legal Metrology
OLA	Ontario Laboratory Accreditation
PNAEQ	Portuguese Program of External Quality Evaluation
QIs	Quality Indicators
QMS	Quality Management System
RIQAS	Randox - International Quality Assessment Scheme
RM	Reference Materials
RMP	Reference Measurement Procedures
RMS	Reference Measurement Services
SANAS	South African National Accreditation System
SD	Standard Deviation
SEQC	Spanish Society of Clinical Biochemistry and Molecular Pathology
SIBioC	45th Congress of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology
TAE	Total Analytical Error
TE	Total Error
TEa	Total Error Allowable
TTP	Total Testing Process

$u_{c\,pre}$	standard uncertainties were combined
UKNEQAS	National External Quality Assessment Scheme
U_{pre}	Expanded Pre-analytical Uncertainty
VIM	International Vocabulary of Metrology — Basic and General Concepts and Associated Terms
WG	Working Groups
WG-H	Working Group on the Harmonization of the Total Testing Process
WG-LEPS	Working Group - Laboratory Errors and Patient Safety
WHO	World Health Organization

1 INTRODUCTION

The International Vocabulary of Metrology (VIM) [1] defines the concept of uncertainty as a non-negative parameter associated with the result of a measurement representative of the dispersion of values that can be attributed to the measurand. From the metrological point of view, a result is not complete unless it's accompanied by its uncertainty.

In Metrology, the notion of uncertainty applied to the results reveals a fundamental importance when interpreting and evaluating those same results. Uncertainty, presenting itself as a quantitative indicator of the quality of the measurement, allows for interpretation and inferences regarding the reliability and confidence in those values. Thereby in areas such as the industry or calibration testing, measurement uncertainty (*MU*) is an accepted and disseminated concept and its application is done according to known and complex mathematical calculations and theoretical models, using probabilities and the law of propagation of uncertainty as the basis for this modelling.

Published in 1995, and reedited in 2008, by a working group of the Joint Committee for Guides in Metrology (JCGM), the GUM: *Guide to the expression of uncertainty in measurement* [2], reveals the considerations and orientations from its member organizations on the matter (BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML). The GUM describes in general terms the calculation of measurement uncertainties, being nowadays a reference strictly followed and widely implemented and cited with emphasis in the field of physical testing, but also in analytical chemistry. However, the specific aspects and conditions regarding the clinical testing in medical laboratories remain excluded, or simply not addressed - not in GUM, or in its derived documents from EA - *European Co-operation for Accreditation* (EA-4/16 – EA guidelines on the expression of uncertainty in quantitative testing) [3], or from the EURACHEM/CITAC (CG4 - Quantifying Uncertainty in Analytical Measurement) [4].

Associate the medical laboratory clinical results with different sources of variation, such as biological variation, pre-analytical and analytical variation, has then been an objective of the clinical laboratories and both medical and technical communities. The aim has been to develop a concept of uncertainty suitable to clinical laboratory results, considering its own conjunctures and specifications, and including some intrinsic characteristics exclusive to them. Late studies have been seeking to answer questions regarding the applicability of the concept of uncertainty in Clinical Laboratories, with the final purpose of reaching a consensus model to estimate Measurement Uncertainty in the Medical Laboratory.

1.1 Medical Laboratory Accreditation:

The International Organization for Standardization (ISO) is a worldwide federation of national standards bodies (ISO member bodies), which develops and publishes International Standards. These standards are carried out through ISO technical committees, with representatives of each member body interested in the subject, for which a technical committee has been established. According to ISO, "*a standard is a document that provides requirements, specifications, guidelines or characteristics that can be used consistently to ensure that materials, products, processes and services are fit for their purpose, safe, reliable and of good quality*". Having International Standards covering almost all aspects of technology, management and quality, worldwide certification and accreditation of laboratories has been guided by its standard's requirements and specifications.

Laboratory Accreditation ensures the existence of the necessary technical skills and competences in the development of the laboratory processes, assuring the quality of the procedures and results. Medical Laboratories Accreditation is foreseen in ISO 15189:2012 - Medical Laboratories — R*equirements for Quality and Competence* [5]. The standard was prepared by Technical Committee ISO/TC 212 - *Clinical laboratory testing and in vitro diagnostic test systems*; and specifies the Quality Management System (QMS) requirements particular to medical laboratories. It was first released in 2003, with a second edition in 2007, which was later replaced, in 2012, by a technically revised third edition, the current version.

It considers that "*medical laboratory services are essential to patient care and therefore have to be available to meet the needs of all patients and the clinical personnel responsible for the care of those patients. Such services include arrangements for examination requests, patient preparation, patient identification, collection of samples, transportation, storage, processing and examination of clinical samples, together with subsequent interpretation, reporting and advice, in addition to the considerations of safety and ethics in medical laboratory work.*"

It is assumed that "*a medical laboratory's fulfilment of the requirements of this International Standard means the laboratory meets both the technical competence requirements and the management system requirements that are necessary for it to consistently deliver technically valid results*".

International Laboratory Accreditation Cooperation (ILAC) presents the ISO 15189 Standard as "*A tool to demonstrate the competence of medical laboratories and ensure the delivery of timely, accurate and reliable results*", which should be used by medical laboratories for development of their management systems and to maintain their own competence; and by accreditation bodies to confirm or recognise the competence of these laboratories through accreditation [6].

It is considered that ISO 15189 covers the essential elements for medical laboratories to demonstrate the quality and competence of their services, which is validated through the periodic assessment of all factors in the laboratory that affect the production of test data, including the technical competence of staff; the validity and appropriateness of test methods, including pre- and post-analytical; the traceability of measurements and calibrations to relevant standards; the suitability, calibration and maintenance of test equipment; the quality assurance of test results, demonstrated by Internal Quality Control (IQC) systems and by the regular participation in External Quality Assurance Schemes (EQAS); also having an acceptable turnaround time as well as the application of appropriate ethical values.

ILAC points out benefits in accreditation process, regarding: the *Healthcare Regulators*, by providing a mechanism for measuring quality improvement and supporting consistency in the quality of care; the *Patients*, confirming that the laboratory has up-to-date-technologies and its procedures and techniques reflect current best practice and that the staff providing the service are competent to undertake the tasks they perform; and for the *Medical Laboratories*, providing opportunity for external perspectives on the laboratory's practice, encouraging the sharing of best practice; stimulating innovation and reducing risk; and providing international recognition.

In 2007, the European Communities Confederation of Clinical Chemistry and Laboratory Medicine (EC4 - Working Group) presented the results of a questionnaire carried out in 2005 to explore the current status of accreditation in EU countries, which was sent to representatives of clinical biochemistry and laboratory medicine societies of EU countries [7]. From the answers of the 19 societies that returned the survey, out of 25, was revealed that the accreditation of medical laboratories in the countries of the EU is mostly carried out, by National Accreditation Bodies (NAB), that work together in a regional cooperation, the European Cooperation for Accreditation (EA), and that some countries have established professional accreditation bodies specifically for medical laboratories, like the Clinical Pathology Accreditation (CPA - UK), which

have incorporated in their requirements all aspects of ISO 15189. The ISO 15189 standard was found to be "*widely accepted in the medical laboratory community*".

The report also revealed that, although the majority of European Accreditation Bodies have a test-by-test accreditation policy, "*many professionals would prefer accreditation of the entire service provided within the actual field of testing (i.e., hematology, immunology, etc.), with accreditation granted if the majority of tests offered within a service field fulfil the requirements of the ISO 15189 standard*".

So, having the discussion about the accreditation scope been an important issue in the last years, both EA and ILAC already acknowledged the possibility of implementing a flexible scope and issued guidelines and recommendations to the purpose [8, 9]. Also, in an additional position paper [10], EA recognized the benefits implementing a flexible scope.

A flexible scope of accreditation, contrary to the fixed list of the methods/tests used under the test-by-test accreditation procedure, would not mention individual tests, but coherent groups of services within a medical field and with similar technical principles with provision of all applicable materials (and matrices such as serum, plasma, urine and blood cultures).

These approaches were recently addressed as alternatives in an editorial piece by Mario Plebani, *et al.* [11] about the accreditation of medical laboratories under the ISO 15189 standard.

A position paper by Marc Thelen, *et al.* [12], on behalf of the Working Group Accreditation ISO/CEN standards (WG-A/ISO) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), recommended the flexible scope for ISO 15189 accreditation, with a guidance based on an approach that has led to successful introduction of the flexible scope for ISO15189 accreditation as intended in EA-4/17 in The Netherlands.

A recently published results (2016), of an EFLM survey initiated in 2014 on the accreditation process in European countries [13], concluded that the ISO 15189 accreditation project has been widely accepted, revealing the efforts of the cooperative work done. The scientific societies and the NABs have provided support and incentives that enabled the laboratories and the laboratory professionals to move toward uniform implementation of the accreditation concept. However, numbers and percentages of accredited medical laboratories are disparate among the EU countries. Although not able to express the real total number of medical laboratories in each country, as there is no authority in each country responsible to give the total number of registered laboratories, the results showed that "*some countries have already reached an almost complete implementation of accreditation*" as "*other countries are still at the beginning of this development*".

29 responses were received, out of 39 countries to whom the delegates of the scientific societies have been sent the questionnaire. All the societies declared the existence of an accreditation process for medical laboratories by a NAB in their country. The accreditation process was declared mandatory by 5 countries (Belgium, France, Hungary, Ireland and Lithuania), with Belgium, Ireland and Lithuania only having a partial mandatory accreditation. Nevertheless, only 7 countries declared to have 50% or more of medical laboratories already accredited (Finland, Ireland, the Netherlands, Sweden, Switzerland, the UK and Belgium), while around 60% of the countries (17 out of the 29 countries) have 15% or below of medical laboratories accredited.

Medical laboratories accreditation processes, in Portugal, are held under the auspices of the Portuguese Institute for Accreditation (*Instituto Português de Acreditação* - IPAC), which applies to the medical laboratories the Portuguese edition of the ISO standard, NP EN ISO 15189:2014 [14], being this process of voluntary participation. IPAC is a recognized member of the existing international organizations of accreditation, as the European co-operation for Accreditation (EA) or the International Laboratory Accreditation Cooperation (ILAC) and the International Accreditation Forum (IAF).

Portugal was not on the countries responding to the solicitation in the mentioned study. However, according to IPAC, since 2008 eighteen Medical Laboratories were accredited by its procedures, within the framework of NP EN ISO 15189. To date (2016), thirteen of which have a valid certificate.

1.2 ISO 15189: Technical Requirements

In Portugal, until 2015, the NP EN ISO 15189:2007 - *Specific Requirements for Quality and Competence* [15], specified the application of uncertainties in medical laboratories, recommending the estimate of measurement uncertainty, considering the need to have such data and to provide to the clients, on request, the results with this information.

The revision and actualization on the ISO 15189:2007, to the version ISO 15189:2012 [5] (NP EN ISO 15189:2014 in Portugal [14]), brought changes to several areas of its application scope, such as ethics, quality and risk management systems, purchase and withdraw of equipment, just to name a few. Moreover, it dealt with qualifications and competence assessment of the laboratory and its technical personnel and focused on the verification of the results, procedures of reporting the results, metrological procedures and traceability. Measurement uncertainty was also contemplated in this extent, having been included additional requirements regarding the MU estimate. On the new edition, in the Technical Requirements Section, when before was a recommendation "*to determine the uncertainty of results, where relevant and possible*" (5.6.2), it is now referred that the "*laboratory shall determine Measurement Uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples*" (5.5.1.4), making it a required requisite, or an obligation.

Subsequently, Medical Laboratories are now required to estimate the measurement uncertainty values associated their results, and to regularly review those estimates, in order to be eligible to apply for Accreditation under this same Standard, or to keep the existing accreditations.

According to *IPAC*, in its Interpretative Guide of ISO 15189 [16], which is still awaiting the actualization following the transition to the NP EN ISO 15189:2014, several approaches or methodologies can be accepted for estimating the value of the uncertainty of results in the Laboratory of Clinical Pathology, as long as they prove to be technically valid and applicable to the methods in the study. It refers that "*due to the generality of the ISO guideline, its application can be done using the sectorial guides adopted or recommended by IPAC, EA, ILAC, Eurachem or Eurolab*".

Therefore, either concept can be applied the "*modelling*" approach, primarily introduced by the GUM, or other more "*empirical*" methodologies based in information from inter-laboratory assays, data from method validation or results of internal quality control for each specific analytical method, gathered from intra-laboratory runs, just having to prove to be technically valid and applicable to the methods in the study. Thus, in Portugal, until the application of the new version of ISO 15189, among other methodologies it has been accepted for a Laboratory to submit to *IPAC* an Accreditation Process for the ISO 15189 Standard, indicating the determination of Total Error (TE), or Total Analytical Error (TAE), to fulfil the requirements of section 5: *Technical Requirements*, of the Standard; particularly on the *Measurement uncertainty of measured quantity values*, which contemplates standard deviation (SD) or coefficient of variation values (CV %) found in IQC and Bias (%) obtained from EQAS. The TE value obtained is evaluated, for each analyte, by comparing to maximum admissible values tables recognized and/or published by internationally scientific organizations [17, 18].

In 1974 Westgard, Carey and Wold described “an approach for formulating criteria that could be used to judge whether an analytical method has acceptable precision and accuracy” [19], introducing the medical laboratory to the concept of Total Error. More recently Westgard remembered that “at that time medical laboratories considered precision (imprecision) and accuracy (inaccuracy, bias) as separate sources of errors and evaluated their acceptability individually” [20]. TE was then recommended as an effort to provide a more quantitative approach for judging the acceptability of method performance. In practice, the authors recommended the determination of TE by combining the estimate of bias (systematic error), from a method comparison study or EQAS and the estimate of precision (random error) from a replication study or from the monthly QC data, using a multiple (z) of the standard deviation (SD) or coefficient of variation (CV); z=2; for a 95% confidence interval or limit of the possible analytic error [20, 21]. Another recent article defines the use of bias as an absolute value (|bias|), being added to the imprecision value adjusted by the coverage factor, which may be set as 1.96 for a two-sided 95% limit, or as 1.65 for a one-sided 95% limit [22].

Regarding the meaning and application of total error, it is explained that “the intended use of TE is to describe the maximum error that might occur in a test result obtained from a measurement procedure. In method validation studies, it provides a measure of quality that can be compared to the intended analytical quality of a test, which can be described in terms of an Allowable Total Error (TEa). TEa is an analytical quality requirement that sets a limit for both the imprecision (random error) and bias (systematic error) that are tolerable in a single measurement or single test result” [21].

This TEa values or specifications can be found, for example, in the “Desirable Biological Variation Database Specifications” (also known as the *European Table* or *Ricos’ Table*) [17], in the “Clinical Laboratory Improvement Amendments (CLIA) Regulations Table” [18] or in the” Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (RiliBaek)” [23].

The TE concept has nowadays more than 40 years of worldwide acceptance and implementation in the medical laboratory. Besides its applicability, the widespread determination of TE also relied in the use of simple mathematical models, applied to easily obtained data, directly from the day-to-day laboratorial work regarding the methods and results validation processes. Using a language that is prevalent in the medical lab, well-known to all pathologists and technicians, it benefiting from having International References determined and accepted for orientation and comparison, as the tables mentioned above.

However, this does not meet the requirements of the GUM or the recommendations on other derived documents for the estimate of measurement uncertainty. Several aspects have been sorted out, advocating for incompatibilities between the two concepts. Being one of the premises in MU that any known bias should be corrected or eliminated, Kallner points out that “a common objection to the TE is that if a known bias is included, why keep it?”, and continues stating that “bias has a sign whereas the imprecision is a characteristic of a distribution” and “therefore the quantities included in the TE are not really comparable [24]. On this point, contrarily to the summation of the squares as applied in the MU estimate, TE considers a linear contribution of method bias, adding the bias value to a probability distribution, and also does not recognizing that “patient results could have other possible outcomes with less error than bias+z·SD” [25]. Moreover, seems to be a model “only valid when imprecision and bias are the only variables involved”, not considering “for biological variation and other evident additional causes of variation” [26].

The question here is not just arguing about the validity of a concept with worldwide implementation over the last few decades in medical laboratories. The decisive point seems to be that it does not correspond to what is pretended with the measurement uncertainty estimate and to the requirements of the accreditation standard.

Arguments vary between affirming the necessity of implementing MU, disregarding the use of TE. It has been stated that laboratories “should cease to define a so-called allowable total error of result, with assessable biases” [27], or that “the concepts of TE and total analytical error represent a loss of information since two independent quantities are linearly added to create a single expression” and so, laboratories “should abandon the total error concept and estimate the uncertainty of the measurement procedure” [28, 29]. Also had been pointed out limitations like “the possibility of an acceptable TE masking unacceptable performance in one of its constituents”, as “an assay with very good precision, may carry a significant bias and remain within the TE goal”, which “may systematically misclassify patients in some settings” [30].

But, more significant than the arguments against TE are the ones favouring MU, advocating that “being a property of the result, also benefits from being able to be combined with different sources of variability (e.g. biological + pre-analytical) to produce uncertainties relevant for clinical decision making” [29, 30].

1.3 MU - Implementation goals:

The intents of ISO 15189 are to make medical laboratory measurements and results transferable, or comparable, on a global basis, stating the idea that a good estimate of measurement uncertainty is necessary for laboratories and their customers to ensure results are fit for purpose and are traceable to international or national standards, to compare results between laboratories and/or specifications, legal tolerances or regulatory limits, to make informed decisions and improve test methods and results.

EA, in EA-4/16 Guidelines [3], state that there “are several advantages linked with the evaluation of measurement uncertainty in testing, although the task can be time-consuming:

- Measurement uncertainty assists in a quantitative manner in important issues such as risk control and the credibility of test results;
- A statement of measurement uncertainty can represent a direct competitive advantage by adding value and meaning to the result;
- The knowledge of quantitative effects of single quantities on the test result improves the reliability of the test procedure. Corrective measures may be implemented more efficiently and hence become more cost-effective;
- The evaluation of measurement uncertainty provides starting points for optimising the test procedures through a better understanding of the test process;
- Clients such as product certification bodies need information on the uncertainty associated with results when stating compliance with specifications;
- Calibration costs can be reduced if it can be shown from the evaluation that particular influence quantities do not substantially contribute to the uncertainty.”

2 MEASUREMENT UNCERTAINTY

The term uncertainty can easily be misunderstood on its applicability, as it in theory represents doubt, in this case doubt about a measurement or a result. In Medical Laboratories the patient's results are presented as the best estimate to the measurand quantity, on the conditions taken, being an undeniable fact that any measurement has a variation associated to it. Recalling the formal definitions on both the VIM and the GUM, by characterising the dispersion of results regarding that single measurement, MU actually indicates how reliable the result really is, providing information on the level of confidence of that same measurement.

2.1 MU - Different Approaches

The different approaches for measurement uncertainty estimate derive from what has been categorised into either "bottom-up" or "top-down" methodologies. Although the examples cited in GUM concentrate on the "bottom-up" approach, the "top-down" approaches are not excluded by the GUM principles.

The fundamental metrological "bottom-up" approach described in the GUM, also called "modelling", is based on estimates of uncertainty, expressed as standard deviations, representing the standard uncertainty associated to each of the individual operations of an analytical procedure. The "bottom-up" approach incorporates all conceivable or potential sources of uncertainty of a method and covering all steps or components of the test, examination or procedure that produce the result in focus. This analytical approach is based on mathematical models formulated to account for the interrelation and interactions of all the

influence quantities that significantly affect the measurand in the measurement process, being the major sources of variability often assessed by method validation studies, experiment or from available information. Applying the law of propagation of uncertainties, the several standard uncertainties considered as influencing the measuring process are then combined to provide, after multiplication by a coverage factor, an expanded uncertainty associated with the specific result.

As the GUM bottom-up approach reveals to be dense and involved in complex mathematical formulae and calculations, it is for many authors unfit to the medical laboratories and not being the method of choice for routine medical testing. The “top-down” approaches, also called “empirical”, on the other hand, start from real data, from results obtained from multiple measurements. Medical laboratories employ quality control schemes and reference materials to estimate, monitor and validate their methods, having available all the data from these laboratory test performance assays (method validation; internal quality control; inter-laboratory evaluation schemes), allowing them to use this information to calculate estimates of the standard uncertainty associated to the results produced by the overall testing procedures/methods [31].

This approach, considered as the most appropriate for routine medical laboratory tests, is generally recognised as a direct estimate of the combined standard uncertainty of the whole procedure, respecting the principles of the GUM approach, but where the calculation is not based on the identification and knowledge of the all the different sources of uncertainty or the use of different mathematical models for the determination of each standard uncertainty.

The “bottom-up” approach indicates the relative magnitude of the various sources of uncertainty, providing a clear understanding of the analytical operations that contribute significantly to the final overall uncertainty, allowing the analyst to identify and focus on improving these operations to reduce the uncertainty associated with test results. However it carries the risk of missing or

undervaluing a contributing factor, or missing a systematic error, which can result in underestimating the measurement uncertainty.

The “top-down” approaches do not identify exhaustively all the independent sources of uncertainty, but assesses the measurement procedure as a whole using data from method validation, from intra-laboratory QC and inter-laboratory studies, or data obtained directly from the manufacturers, considering the random and systematic components of uncertainty and assuming the premise that all sources of variation or uncertainty affecting the final result of a measurement are reflected and contemplated. It is reasoned that the use of such data, generated over several months, will maximise the probability of including all potential contributions to uncertainty in estimates of measurement uncertainty. To medical laboratories, the “top-down” approaches avoid the risks of the “bottom-up” approach and, being its bases known and fully integrated in the laboratories routine procedures, they end up to be intuitive and possibly much more simply to implement.

Again, according to the GUM, “the standard uncertainty of the result of a measurement, when that result is obtained from the values of a number of other quantities, is termed combined standard uncertainty and denoted by u_c. It is the estimated standard deviation associated with the result and is equal to the positive square root of the combined variance obtained from all variance and covariance components, however evaluated; using what is termed in this Guide the law of propagation of uncertainty” (3.3.6).

For example, if a measurement is considered to have three identified and measurable sources of uncertainty, a, b and c, for which the three standard uncertainties, or relative standard uncertainties, u_a, u_b and u_c have been determined, then the combined standard uncertainty u_c for the measurement is given by equation (2-1):

$$u_c = \sqrt{u_a^2 + u_b^2 + u_c^2} \qquad \textbf{(2-1)}$$

2.2 Sources and Types of Uncertainty:

For any of the approaches, each source or component of uncertainty can be assessed and calculated by different methods, depending this determination on the nature of the information/data available on the component itself, which can be classified as type A and type B. According to the Guide to the Expression of Uncertainty in Measurement (GUM) [2]: “the purpose of the Type A and Type B classification is to indicate the two different ways of evaluating uncertainty components and is for convenience of discussion only; the classification is not meant to indicate that there is any difference in the nature of the components resulting from the two types of evaluation. Both types of evaluation are based on probability distributions and, the uncertainty components resulting from either type are quantified by variances or standard deviations” (3.3.4). It is important to highlight, “these categories apply to uncertainty and are not substitutes for the words random and systematic” (3.3.3).

So, standard uncertainty can be evaluated by two different methods; “Type A: method of evaluation of uncertainty by the statistical analysis of series of observations” (2.3.2); “Type B: method of evaluation of uncertainty by means other than the statistical analysis of series of observations” (2.3.3). Type B “is evaluated by scientific judgement based on all the available information on the possible variation of the measurand.

The pool of information may include:

i. previous measurement data;
ii. experience with or general knowledge of the behaviour and properties of relevant materials and instruments;
iii. manufacturer’s specifications;
iv. data provided in calibration and other certificates;
v. uncertainties assigned to reference data taken from handbooks” (4.3.1).

When different sources of uncertainties are assigned to a measurement standard uncertainty, they can be of both, type A and type B. These standard uncertainties, or relative standard uncertainties, are combined according to its propagation law, or individual properties. Being the uncertainty sources considered independent (not correlated), individual standard uncertainties are combined using summation in quadrature, also called root sum of the squares.

As mentioned by Badrick, *et al.* [32], when clarifying the concept of uncertainty and its applications in the medical laboratory, this variation or variability of the final result have five major components: pre-analytical factors, intra-individual variation, assay imprecision, operator differences and pathological processes.

Technically, the pathological processes are the ones that in fact concern the medical practice and the clinician requesting the tests. It is this source of influence over a test result that must be valued in a patient's evaluation. In consequence, the remaining components, or sources of variability, are the ones that need to be assured that are minimized and assessed in the laboratory, so they can be properly weighted and considered in the process of analysing the results presented.

White and Farrance consider two major sources of uncertainty contributing to the total uncertainty of measurement of a routine quantitative diagnostic method. They characterize them as the "uncertainty associated with the value of a test result due to the random errors that normally occur when conducting the testing procedure" and the "uncertainty associated with the numerical value assigned to the measurand present in the calibrator material used in the routine method", which "should be estimated by the commercial supplier of the calibrator" [33].

So, on the analytical component, different variation factors affect the measurement accuracy. Measurement accuracy is defined by the VIM as the “closeness of agreement between a measured quantity value and a true quantity value of a measurand”, which is related to the conceptions in the GUM, since the objective behind the concept of measurement uncertainty is to determine an interval of reasonable values around the result where that true value of the measurand lies, i.e., “to estimate the dispersion of the values that could reasonably be attributed to the measurand”.

“A quantitative estimate of the accuracy of a result is essential to define the degree of confidence that can be placed in it and the reliability of the decisions based on such result. Such parameter is the measurement uncertainty “ [34].

Thus, the concept of measurement uncertainty encompasses the combination of the variation components that affect the accuracy of a result, the qualitative performance characteristic of a measurement mentioned above.

Mendito, *et al.* noticeably explain the concepts and the relation between the different performance characteristics [34], as illustrated on

Figure 1. Assuming that variation sources influence a measurement result in both random and systematic ways, these concepts are directly related to accuracy, which includes the respective qualitative performance characteristic regarding random and systematic variation, precision and trueness.

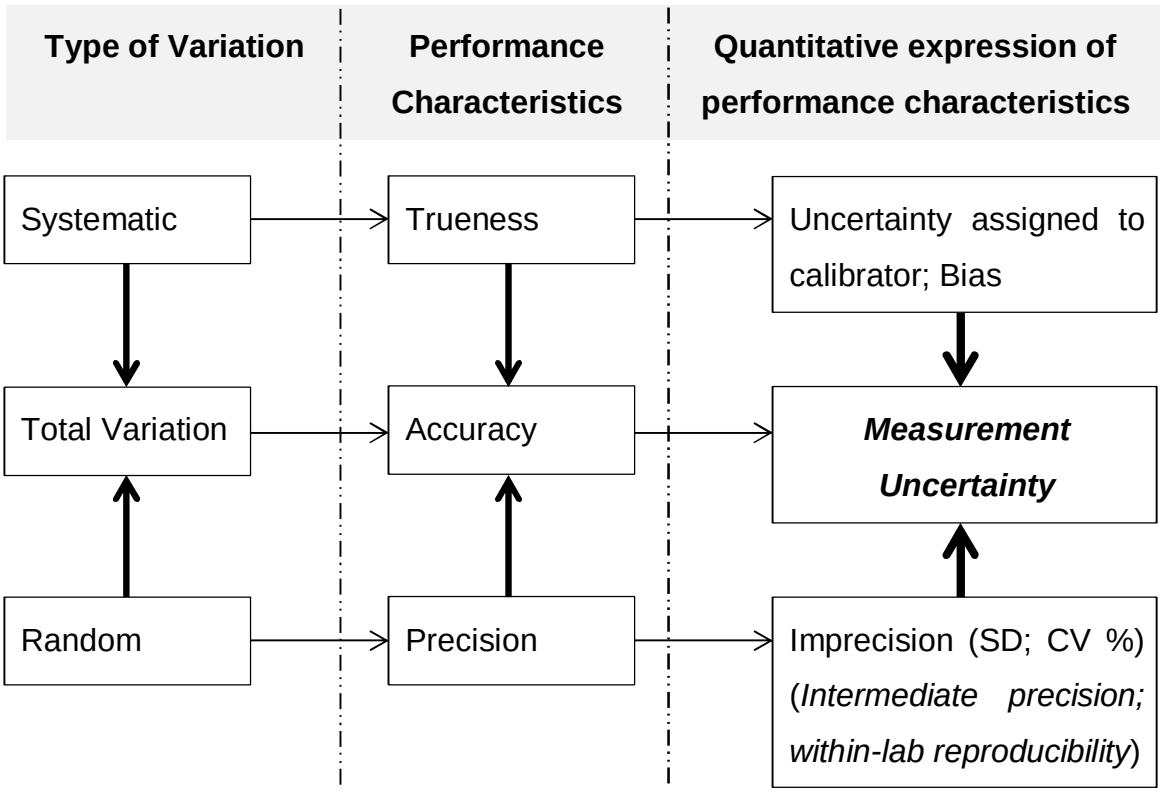


Figure 1 - Relationships between type of variation, qualitative performance characteristics and their quantitative expression. (Adapted from Mendito, *et al.* [34])

The VIM defines precision as the “closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions”, stating that “measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement”. In medical laboratories the concept can be translated to the performed IQC, where those long term determinations within the same measurement procedure, which can include different conditions, involving changes as new calibrations, different reagent lots or different operators, correspond to the intermediate precision determination, sometimes also known or addressed as “within-lab reproducibility” [35].

IQC is employed to assess the day-to-day testing and to technically validate test results on an everyday basis routine, monitoring the whole procedure's imprecision. This is then accepted as the estimate of the intermediate precision of the measurement and considered to be the ideal source of data to the analytical random variation component, when determining measurement uncertainty [24, 31, 35-37].

Trueness is defined as the "closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value". "Measurement trueness is inversely related to systematic measurement error", whereas bias is presented as the "estimate of a systematic measurement error" [1].

The variation associated with the calibrators used in the medical laboratory methods relates directly to trueness. The GUM, as the principles of measurement uncertainty, aims to the characterization of unbiased results, advocating the minimization, elimination, or correction, of any known bias. This minimization, and particularly its assurance, can only be accomplished through measurement's metrological traceability, as stated by Dybkaer when considering that "the necessary anchor for the trueness of a measurement procedure is obtained by strict metrological traceability of result, based on a calibration hierarchy" [27]. The VIM describes metrological traceability as the "property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty", which "requires an established calibration hierarchy". Calibration hierarchy is described as the "sequence of calibrations from a reference to the final measuring system", where the "measurement uncertainty necessarily increases along the sequence of calibrations".

Metrological traceability and calibration hierarchy concepts are understood and accepted in the medical laboratory scientific community, taken not only as the basis of results reliability and comparability, but as essential requirements to verify and fulfil in order to achieve the necessary and expected accuracy/trueness in medical laboratory measurements [38-41]. The relation between metrological traceability and measurement uncertainty is addressed and sustained in the ISO 17511standard [42], as illustrated in Figure 2, which together with another standard, the ISO 18153 [43], gives support to the European Union Directive 98/79/EC, on In Vitro Diagnostic (IVD) Medical Devices [44]. The EU Directive obligates manufacturers operating within the EU to assure and demonstrate the metrological traceability of their products, when used in conjunction with other components of their analytical system (platform and reagents), stating that ‘‘the traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order’’.

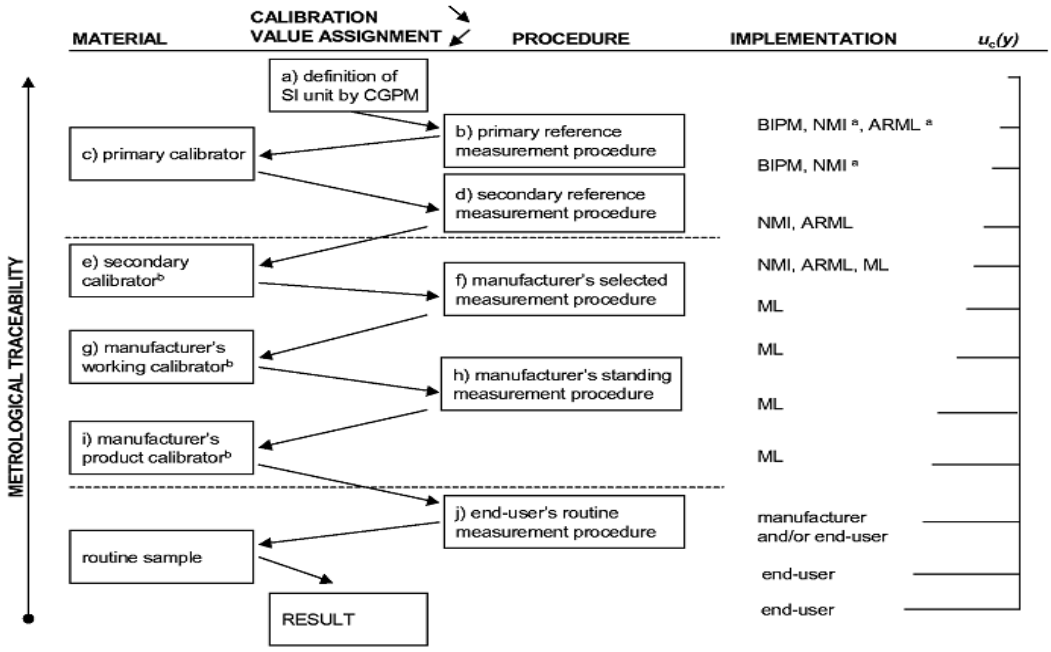


Figure 2 Relation between Metrological Traceability and Measurement Uncertainty (*from ISO 17511:2003 Standard* [42]).

In addition to the previously mentioned standards, ISO provided further guidelines and requirements regarding the content and presentation of reference measurement procedures; the description of certified reference materials and the contents of its documentation and the requirements for reference measurement laboratories in laboratory medicine, respectively in the standards ISO 15193, ISO 15194 and ISO 15195 [45-47], which in general are applied directly to manufacturers and reference measurement laboratories, in the matters of traceability and standardization.

The mentioned standards, which provide international agreement upon the requirements constituting higher-order reference materials and methods, serve as bases for the tasks of the Joint Committee for Traceability in Laboratory Medicine (JCTLM). The JCTLM was created in 2002 under the auspices of the Bureau International des Poids et Mesures (BIPM), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC), in response to the necessity of clarification on which reference materials and methods should be used by manufacturers and reference laboratories to anchor their assays [48]. Following on its own *Mission Statement*, JCTLM aims to support world-wide comparability, reliability and equivalence of measurement results in Laboratory Medicine, for the purpose of improving health care.

The goal of JCTLM is to identify appropriate reference materials (RM) reference measurement procedures (RMP) and reference Measurement Services RMS), which is achieved through the operation of two working groups (WG): WG1 assesses submissions for RM and RMP and WG2 assesses submissions for RMS.

According to Armbruster [48], medical laboratories perform analysis for about 400-1000 different measurand, with only 10 % having well-recognised reference materials and methods exist and being traceable to the SI unit and others having either a reference measurement procedure or a reference material but not both. Referring that, to date, JCTLM identified reference materials and/or methods for about 120–150 analytes, he denotes that in the promotion of assay standardisation and global harmonisation in the clinical laboratories “the pace, completeness, and success of this movement depends on cooperation among a variety of entities, including professional societies, regulatory bodies and other governmental agencies, industry, and individual clinical laboratories” [48].

Recently, the Journal of the International Federation of Clinical Chemistry and Laboratory Medicine (eJIFCC) dedicated its February (2016) issue to the “*Harmonization of Clinical Laboratory Test Results*”, in which Tate defined harmonization as the “ability to achieve the same result (within clinically acceptable limits) and the same interpretation irrespective of the measurement procedure used, the unit or reference interval applied, and when and/or where a measurement is made” [49]. As previously was affirmed, it is here reinforced that this achievement will englobe coordinate action, involving national and international associations and scientific societies and integrating the total testing process, with focused attention on the pre-analytical, analytical and post-analytical phases [49, 50].

To promote harmonization among the 40 European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) member societies the Working Group on the Harmonization of the Total Testing Process (WG-H) was recently established. The aims of the WG are: “1) surveying and summarizing national European and pan European harmonization initiatives; 2) promoting and coordinating the dissemination of especially promising harmonization initiatives among the EFLM member societies; and 3) taking initiatives to harmonize nomenclature, units and reference intervals at a European level” [51].

Based on the results of its first questionnaire, surveying the status of various harmonization activities among the European laboratory medicine societies, some activities promoting the dissemination of best practice in blood sampling, sample storage and transportation, in collaboration with a Working Group on the Pre-analytical Phase, are already being promoted, as there are some initiatives to spread to all the European countries the use of SI units in reporting [51].

Similarly, the American Association for Clinical Chemistry (AACC) has created an International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR), working with both its partners, domestic and international [52]. The consortium intends to address the key issues identified in an international conference promoted by the AACC, on traceability and harmonization in laboratory medicine. In order to organize the global harmonization efforts the infrastructure is to address: 1) prioritizing measurands by medical importance, 2) coordinating the work of different organizations, 3) developing technical processes to achieve harmonization when there is no reference measurement procedure or no reference material and 4) promoting surveillance of the successes of harmonization [52].

There is also an important role reserved to the industry and to manufacturers on the road to harmonization, as addressed by Armbruster that states the imperative for a close coordination between industry and professional bodies, particularly in terms of coordinating and prioritizing projects leading to product development, which are largely conditioned by time and costs. While attributing major importance to traceability to reference measurements, some concerns are identified regarding “the cost of harmonization which includes physician education, patient safety and investment in product redevelopment”, according to which the process must be considered and “carefully weighed to understand the benefit of harmonization” [53].

2.3 Measurement Uncertainty in the Medical Laboratory

In the medical laboratory, it has now been given a critical role to this concept and to the estimate of measurement uncertainty. Mandatory, regarding the process of laboratory accreditation, the implementation of this new tool, once adapted and put to function, will allow define, quantify and evaluate the quality of its procedures and results, assuring the reliability of the whole laboratory process, as well as of the values that it produces, making evidence of the laboratory quality and competence.

Therefore, the decisive step towards this reality implies not only consensus and specific information or regulation, on the determination of values of measurement uncertainty directly applied to the medical laboratory, but also its wide dissemination and discussion, for a correct understanding by all the involved and a gradual integration of the concept. This will promote and encourage an adequate appreciation on the interpretation of its outcomes and applications in the laboratory, regarding clinical diagnostic and patient monitoring. Only exhaustively studying the problematic in a comprehensive way will allow to assess methodological approaches and to set future guidelines [54]. Questions about the very acceptance of physicians, confronted with this information, as well as its effect in the final clinical decisions are still unanswered. Will this process serve to improve the efficiency and effectiveness in the evaluation, interpretation and diagnosis [55]? The problems associated with uncertainties measurement will continue until their application and understanding extends to most laboratories at national and worldwide levels, being an essential requirement the involvement of the medical community (clinical pathologists and physicians) and its associations, as well as the medical laboratory technical staff (Biomedical Laboratory Scientists).

Meanwhile, this application to medical laboratories has been under discussion since the inception of the GUM, and there are authors who consider it inadequate or unfit for laboratory testing in health, and do not recommend its implementation [56]. Having been in the genesis of Total Error, and being one of the critics of the GUM and ISO 15189 requirements regarding measurement uncertainty, James O. Westgard recently affirmed: “Given the difficulties in implementing the original recommendations in the Guide to the Expression in Measurement (GUM), more guidance is clearly needed if laboratories are to characterize the uncertainty of their many different measurement procedures.”[57].

Nonetheless, as addressed, in the medical laboratory, the concept and the concern with the estimation of uncertainty is relatively recent and there is no standardized or globally implemented procedure to calculate the measurement uncertainty associated to its results.

Being an international perception and understanding that the classical modelling approach of the GUM is not the fittest for medical laboratories application, many consider that its direct application is not feasible at all.

Several models and different approaches have been studied over the last years [58-63], not having yet resulted a consensus on its implementation. Although encouraged, the determination of measurement uncertainty is not a common practice in the laboratory tests in health, raising yet too many questions, analytical and clinical [54, 55], having its application been subjected to several contradictory arguments since the publication of the GUM [63-65].

Applied research studies will enable to substantiate the generalization of the estimative/measurement of uncertainties in medical laboratories, contributing to spread this practice, grounding the reasons to its application and for the association of its values to the laboratory results in Clinical Pathology, which are nowadays, in modern medicine, the foundations of clinical decision-making and medical practice, with estimated 70-80% influence in decisions affecting diagnosis or treatment [66-69].

The sources of uncertainty of a medical laboratory result are many and varied but, focusing on “Top-Down” approaches, can all be embedded under the following broad categories: Biological Variation; Pre-Analytical Variation; Analytical Variation.

In the literature are advocated several different combinations on this categories in order to reach a final estimate of measurement uncertainty. Some models value one category more than another; some emphasize different points on the process, not contemplating others; some even ignore or just discard one category, devaluing its contribute or considering that it is being accounted somewhere else along the way.

Thereby, even when following a “Top-Down” approach, there can be found different models and procedures being applied to assess measurement uncertainty. There are reductive models, that directly transfer the concepts of Total Error to a process of combination of data from imprecision and bias, using this data as values of standard uncertainties to obtain the combined uncertainties, that are expanded to the final uncertainty of the results [70, 71], these arguments are also stated by Panteghini in an editorial of Clinical Chemistry and Laboratorial Medicine (CCLM), the official journal of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [72]; There are some that take into account other factors, like the uncertainty associated to calibrators, internal QC or certified reference materials used in intra- and inter-laboratory test assessment schemes [73, 74]; Others argue about the inclusion, exclusion or correction of *bias*, or recall and include pre-metrological factors and variability [31, 75, 76].

3 Framework – Laboratory and Analytes

3.1 GNÓSTICA - Medical Laboratory

Gnóstica – Medical Laboratory (GN) was founded in 1986 and develops its activity of providing health services in the area of Clinical Analysis, which have always been done based on adequately controlled processes with the purpose of obtaining high quality results.

In 2003, the awareness that the practice of quality services should be regulated and controlled by European and National Standards led to the implementation of a Quality Management System, using as reference the Portuguese Edition of the ISO 9001:2000 (NP EN ISO 9001:2000). Thus, GN was certified by the National Agency, the Portuguese Quality Institute (IPQ), within the scope of NP EN ISO 9001:2000, by the end of the same year.

Figure 3 - Gnóstica Medical Laboratory (GN): A) Building facade; B) Waiting room; C) Sample collecting room.

The prospect of progress towards Total Quality, based on a continuous improvement philosophy, encouraged the ambitious process of accreditation by the NP EN ISO 15189:2007. The procedures were initiated in 2008, with the Portuguese Institute of Accreditation (IPAC), having the Laboratory obtained its Accreditation Certificate by the Standard NP EN ISO 15189:2007 in 2009 (Appendix D), for 86 tests. The following year these were extended to a total of 96 accredited tests.

Fulfilling the requirements of ISO 15189, besides the methods validation protocols and the implemented IQC System in the day-to-day results validation, GN meets the external validation requirements by participating in several EQAS, namely the Portuguese Program of External Quality Assessment (PNAEQ), from the reference laboratory of the National Health Institute – Dr. Ricardo Jorge (INSA); Randox - International Quality Assessment Scheme (RIQAS); Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC); National External Quality Assessment Scheme (UKNEQAS).

Figure 4 - Gnóstica: external collection sites in the Algarve region.

GN stands as the only Accredited Medical Laboratory in the south of Portugal (regions of Alentejo and Algarve). It currently have 41 external collection sites in the Algarve region, supporting the central laboratory, which registered a total volume of 962.822 tests performed last year (2015) for a total of 104.820 patients.

Figure 5 - Roche's auto-analyser cobas®6000: 1) Core unit; 2) C501 module; 3) c601 module.

The clinical chemistry laboratory is equipped with Roche's system. The auto-analyser *cobas® 6000* is constituted by two different modules, the *cobas® c501* and the *cobas® e601*, which can be assembled in 7 different module combinations, with up to 3 modules in one core unit, capable of running more than 160 assays on a single platform, as presented on Appendix E. The *cobas® c501* is a photometric measuring unit (including ISE) and the *cobas® e601* unit is based on ECL (Electrochemiluminiscence) Technology. All of the analytes in the study were determined on the cobas® c501 module, using the manufacturers' own and recommended methods and reagents, as well as calibration and control materials.

Figure 6 - Roche's auto-analyser cobas®6000 (side view)

3.2 Analytes: decision-making

Before starting the first practical approach and implementation of the different methodologies for the determination of measurement uncertainty (MU) in the medical laboratory, it was necessary to take some decisions on the subject, in order to narrow the options and to define which analytes to include in the study.

The list started with a list of 35 Clinical Chemistry possible analytes, which regarding the determination methods practically all were photometric based (only a few tests on the list were potentiometric). Considering the method performance for each analyte, were found 20 analytes with a result of *excellent*, 4 with *good*, 4 within the *acceptable*, 6 that are *non-conforming* and 1 with no references. This evaluation based on comparing the Total Error for each analyte with the values from the "Desirable Biological Variation Database Specifications" [17]. (Should be noted that the non-conforming methods all proved to be inside the specifications of the "CLIA regulations table" [18], which also establishes guidelines for analytical quality requirements and acceptable performance criteria for medical laboratories, as stated before, being this way considered that all the methods fulfil the minimum desirable quality specifications.)

Some studies on MU were found in the literature which, despite the different approaches being used, addressed some of these analytes and provided not only different perspectives on the theme, but also comparison data for future results to be obtained. Glucose was the most studied analyte, with several references [31, 59, 60, 70, 74, 76-79], followed by Creatinine with [70, 79-82]. Could also be found more than one reference to seven other analytes, being Albumin, Calcium, Alkaline Phosphatase, Potassium, Magnesium, AST/GOT and ALT/GPT.

Weighted the factors, and also after discussing the subject with the Technical Board at GN, the decision fell on *Glucose*, *Creatinine* and *Total Cholesterol*. Were chosen two different analytes already addressed in other studies, and so with data to compare if necessary and suitable, as well as an analyte with fewer studies on this matter (Total Cholesterol). This would contemplate two analytes with excellent quality performance, as well as one non-conforming, accordingly to the mentioned criteria (Creatinine), with three different test methods (Creatinine: kinetic method; Glucose: enzymatic UV method; Total Cholesterol: enzymatic colorimetric method).

Although it was possible to starting with more analytes, that can also be done afterwards, as the intended is a future enlargement of the study, taking advantage of the framework of this work in order to implement this methodology to the whole laboratory.

Nevertheless, the aim of the current study will always be to test and validate an approach and formula to estimate MU, able to be implemented in the laboratory and accepted by IPAC in the NP EN ISO 15189:2014 accreditation process.

4 AIMS and OBJECTIVES

4.1 Aims

Based on the presented presuppositions and given the lack of studies on estimation of measurement uncertainty in Portugal, especially on its application in the medical laboratory, it is intended to study the subject, in an exercise exclusively dedicated to methods of quantitative analysis, particularly in the field of clinical biochemistry.

In order to achieve this, different approaches and formulae for the estimate of measurement uncertainty will be tested and compared, considering its application in the context of the medical laboratory. The appraisal on the applicability and quality gains of the different models will enable focused discussion and considerations, allowing inferring or even concluding about their practical validity.

In the course of the study, each formula and its outcomes will be appreciated and valorised bearing in mind the diverse sources/categories of uncertainty identified with direct and measurable influence on the final result. For instance, will be considered the inclusion/exclusion of Bias or of the uncertainty values assigned to the calibrators, as well as the assessment and quantification of the uncertainty of the pre-analytical phase and its variables; always prioritizing the suitability of each model and its applicability in the laboratory daily routine.

Hopefully, this study will contribute to definition and optimization of a generalized model for the estimation of uncertainties associated to results in the clinical laboratory, subsequently providing easy access to it, allowing its wide application in Medical Laboratories and enabling the standardization of determination models and the comparison of estimated values. To achieve a

model that can gather consensus means to achieve a model that fits the characteristics of medical laboratories and meets their specific realities. This will allow a practical application of uncertainties measurement and its dissemination, liberalizing this procedure and, eventually in the future, the emission of results in clinical laboratories along with their associated uncertainty values. But, this is yet another subject held in great discussion, regarding the medical laboratory scientific community, but also and more importantly, the "costumers" of the laboratory, not only the users, but particularly the clinicians, situation that will require joint actions on the education of the involved. .

The ultimate objective will be to ensure a formula that fulfils the mandatory requirements for laboratories accredited within the NP EN ISO 15189:2014 standard, regarding the estimate on measurement uncertainty requisite, complying with the regulations of the Portuguese Accreditation Institute (IPAC).

Consequently it aims to enable the Accreditation of Medical Laboratories in Portugal, in regards to this particular requirement, while meeting the principles concerning the measurement uncertainty estimate when applied to medical laboratory and clinical purpose.

4.2 Objectives

In a general overview, the various landmarks of the progress until the final goal can be easily identified and summarized. This set of milestones, described ahead in more detail, will be composed by:

1. Characterization of the laboratory's path and evolution towards its total quality goals. Retrospective analysis of data from the Quality Management System (QMS), considering the progress in the quality

indicators (QIs) outcomes alongside the implementation of the Certification and, later, Accreditation processes;

2. First approach to the measurement uncertainty estimation, comparing the application of two different formulae; (2011)
3. Study focused on the pre-analytical phase, aiming to quantify the laboratory's uncertainty associated to the pre-analytical variation. The pre-analytical variables represent a category, or source, of measurement uncertainty considered in one of the formulae implemented on the current study, in which was previously used data from the literature.
4. New application of the previous formula, with the input of the obtained data regarding the pre-analytical uncertainty, directly associated to the specific conditions, materials and procedures of the laboratory in question. (2013)
5. MU Estimate – Gnóstica's Accreditation process and audit: presentation of a preliminary MU report, by the time of the first IPAC's audit, regarding the accreditation process at Gnóstica, under the scope of the new a preliminary report is intended to be presented, *NP EN ISO 15189:2014*.
6. Corrections and adjustments to the MU formula, considering the previous results and state of the art publications on the subject. Application of the final formula. (2015)
7. Working spreadsheet to implement in the laboratory's own quality procedures and routines providing an instrument that is credible and fit for purpose as well as user friendly and perfectly able to be integrated as one of the QMS assessment tools, giving response to Accreditation guidelines and standards and to its requirements on evidences of quality for the laboratory's analytical process and technical competence.

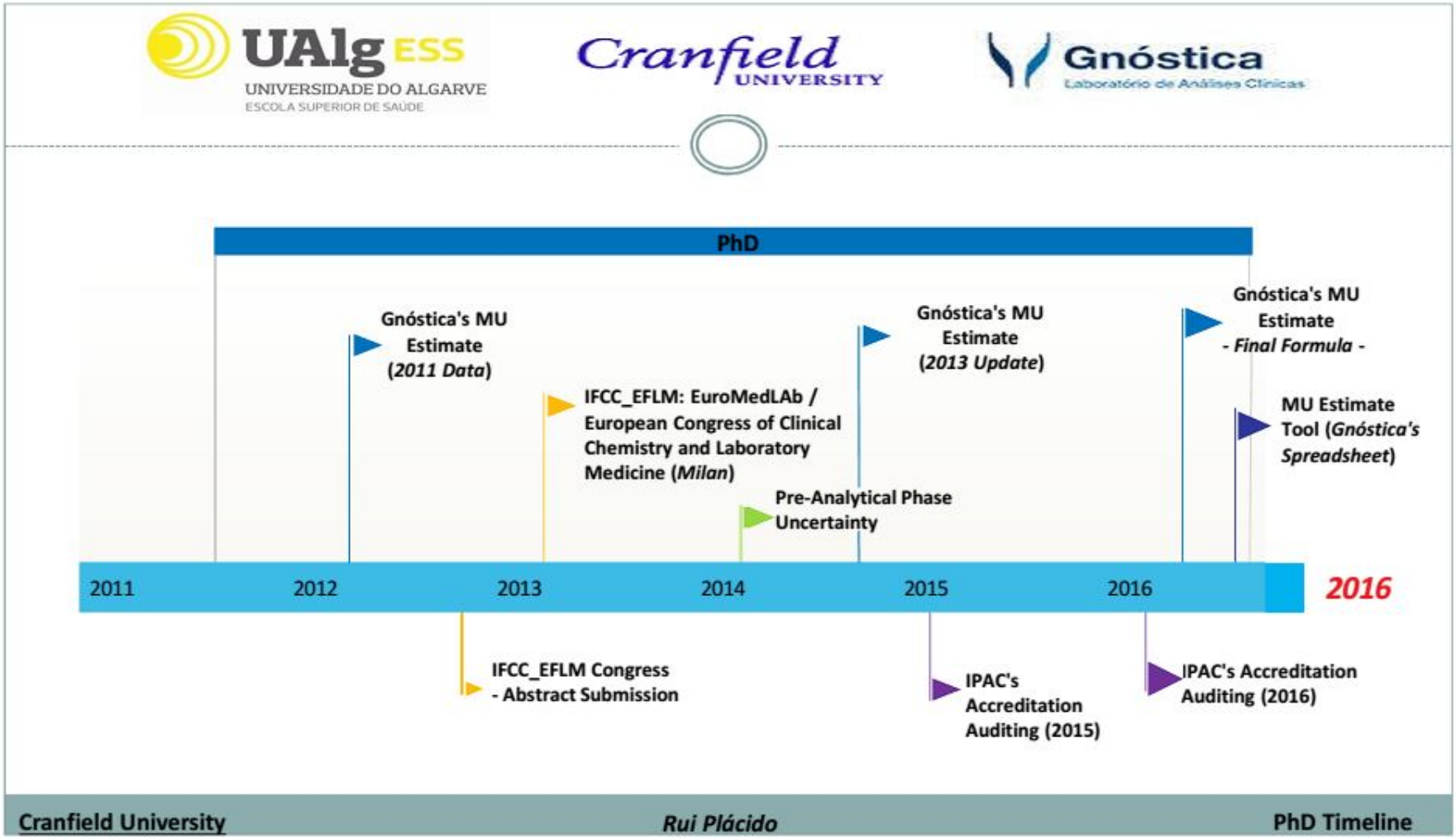


Figure 7 - Estimating Measurement Uncertainty in the Medical Laboratory - PhD Timeline

5 METHODOLOGY

5.1 Quality Indicators

An implemented quality management system intends not only to assure the quality of the processes and of the results, but it aims to support a full quality service, having the purpose of driving the whole structure towards this principle.

When an organization develops its work based on a quality philosophy, with well-defined quality policies, quality improvement must be implicit in every aspect of the day-to-day practice and has to make part of the routine procedures and working habits off all the staff.

Accreditation under ISO 15189 standard [5], in its Management Requirements, defines the need of a continual improvement concern and the demonstration of effective measures to identify opportunities for improvement, with the establishment of goals in all areas of the laboratory activity. This encompasses the pre-analytical, analytical and post-analytical phases, which should be assessed and subject of management reviews in order to compare the laboratory's performance and to promote the development and execution of corrective and preventive actions.

In order to accomplish this, the laboratory "shall establish quality indicators to monitor and evaluate performance throughout critical aspects of pre-examination, examination and post-examination processes". These quality indicators, which shall be periodically reviewed to ensure their continued

appropriateness, enable monitoring non-examination procedures, providing valuable management information, allowing to systematically evaluating the laboratory's contribution to patient care [5].

With the objective of characterising Gnóstica Laboratory, particularly in regards of its general quality outcomes, the most reliable source of information are its performance indicators, which allow following the development and improvement of some quality references. This monitoring process gives direct information on the laboratory's performance and evolution in all areas, not restricted to the analytical results, demonstrating the growth of the laboratory in consequence of its investment in matters of quality policies.

This study started with a retrospective analysis of the laboratory's Quality Management System data, considering the evolution of several QIs during the implementation processes of NP EN ISO 9001:2000 Certification first, and afterwards Accreditation by the NP EN ISO 15189:2007. This aimed to evince the advantages of each of these processes, with a demonstration of the benefits and gains of choosing and implementing quality policies in a laboratory and of looking towards the offer of better services, client satisfaction and high quality results delivery.

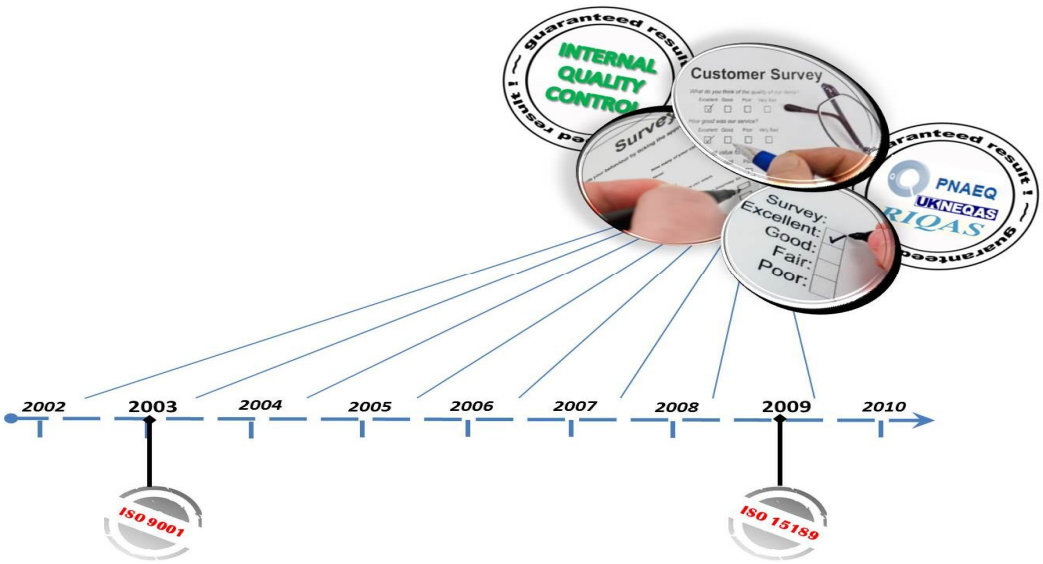


Figure 8 - Timeline from the process of Certification (NP EN ISO 9001:2000) to Accreditation (NP EN ISO 15189:2007) of Gnóstica Medical Laboratory

To evaluate the results of the QMS along the upgrade from Certification to Accreditation, the main Quality Indicators from Pre-Analytical, Analytical, and Post-Analytical phases were analysed for the period from 2003 to 2010.

The data was accessed and collected from the laboratory's QMS annual reports. The QMS review is an essential step of the quality chain and procedures, being the annual reports and the respective review meetings, for results analysis, requirements of the implemented quality standards.

Aiming to a total quality management and continual improvement, these documents gather information relating to all the laboratory's services and practices. Procedures from all the phases of the laboratorial process are here considered, using the internal daily routine quality records and an anonymous questionnaire distributed annually to laboratory users. The QMS keeps records of key quality and performance indicators, identified and assessed for each one of these phases, right from the user's first contact and registration, until the delivery of the results.

Thus, being a part of the laboratory's quality policy and procedures, the performance of Gnóstica Laboratory, the conditions and services offered to its clients, as well as their satisfaction and quality perception, are assessed by the results of the anonymous questionnaire, which is randomly applied every year to the users. A grand total of 3477 completed surveys were analysed, during this period.

Referring to the pre-analytical phase the waiting time and the registry errors regarding user's data/identification or requested tests (such as wrong names or the change/absence of analyses from the request) were analysed.

On the analytical phase, to follow the evolution of the QIs, were controlled the numbers of repeat testing and evaluated the results from the External Quality Assessment Schemes.

Finally, were considered the errors detected on the post-analytical phase and also studied the records of the execution of the tests, with the turnaround time being assessed in terms of fulfilment of expected delivery date and the rate of results delivered in the same day of the request/sample collection.

The information on the Management System was also considered, to analyse the evolution of the Laboratory regarding the number of users/clients and numbers of tests/analysis through the years.

The results of this study were presented in the IFCC-WorldLab-EuroMedLab Congress; 21th International Congress of Clinical Chemistry and Laboratory Medicine; 19th IFCC - EFCC European Congress of Clinical Chemistry and Laboratory Medicine; 8th Annual Meeting of the German Society of Laboratory Medicine and Clinical Chemistry; in Berlin (2011). The work was presented as a poster communication titled "*Quality Indicators in a Clinical Laboratory: from Certification to Accreditation*". (Appendix B)

5.2 Measurement Uncertainty in the Medical Laboratory (I)

5.2.1 Application of two different MU estimate formulae

The experimental design for the first practical incursion in the study starts by adopting/defining two different measurement uncertainty estimating models, both according to Top-Down reasoning, followed by the application of the two formulae in the ISO-15189 Accredited Clinical Laboratory – Gnóstica Medical Laboratory. In each of the approaches, to achieve a final value of MU, the different dimensions of measurement uncertainty were then considered. Subsequently, the different sources or, better saying, categories of uncertainty contributed with a partial value to the final estimate.

After assessing the several components of uncertainty in the procedure, which were considered as having a relevant contribution to the result, these intermediate values were converted to relative standard uncertainties to be combined and finally multiplied by a coverage factor, to give the final expanded uncertainty (*U*).

Being both models based on the laboratory's QMS data (year: *2011*), one restrictedly used the laboratory's IQC data and EQAS results, for imprecision and bias, adapting the concept of TE and combining the considered variation components in a squared model [83]. The second approach added a different perspective; with different variables being taken into account. Focusing on the uncertainty sources and on its influences along the different phases of the measurement process, was considered the data associated to the calibrating materials, provided directly by the manufacturers, being also introduced the well-known concept, often neglected in its quantification, of pre-analytical variation.

Therefore, were considered and applied Models A (MU-A) and B (MU-B). Each estimate followed a sequence of different basic steps that guide the implementation of the model, allowing a structured determination and a well-defined evaluation of MU, as described below:

MU-A) A six step uncertainty calculation model was used to evaluate MU [31, 35, 70]:

- *Step 1:* Defining the Measurand;
- *Step 2:* Imprecision of Measurement;
- *Step 3:* Bias of Measurement;
- Step 4: Converting the components to a standard uncertainty;
- *Step 5:* Calculate combined standard uncertainty;
- *Step 6:* Calculate Expanded uncertainty / Expression of MU.

The complete estimate is supported by the application of equation (5-1) to calculate the Expanded MU (with k=2, for a coverage probability of 95%).

$$U = k \cdot \sqrt{\mathrm{u}Imprecision^2 + \mathrm{u}Bias^2} \quad \textbf{(5-1)}$$

Considering the day-to-day imprecision and the bias value, respectively as the random and systematic variation components.

MU-B) MU estimate by calculating the combined uncertainty (u_c) using equation (5-2):

$$u_c = \sqrt{\mathrm{u}_{Imprecision}{}^2 + \mathrm{u}_{Cal}{}^2 + \mathrm{u}_{Pre}{}^2} \quad \textbf{(5-2)}$$

In which was considered:

- i) day-to-day imprecision;
- ii) uncertainty of the calibrator assigned value;
- iii) Pre-analytical variability.

Subsequently, the same coverage factor was then applied to obtain the Expanded Uncertainty (*U*), as demonstrated by equation (5-3).

$$U = k \cdot u_c \quad \textbf{(5-3)}$$

With the previously obtained combined uncertainty being multiplied by the factor k=2, for a coverage probability of 95%.

Only with the purpose of comparison, was also presented and considered the values for Total Error, obtained from equation (5-4), where the estimate of bias is combined with the estimate of the methods imprecision (CV_A) multiplied by a factor (*z*=2), also for a confidence interval of 95%.

$$TE = \text{bias} + z \cdot CV_A \quad \textbf{(5-4)}$$

Again considering the day-to-day imprecision and the bias value, respectively as the random and systematic variation components.

5.2.2 Material and data

As mentioned before, in the different approaches and MU estimates the data from the laboratory's QMS will be used, namely the data from the IQC and the EQAS. Whenever possible and/or relevant, will also be used specifications and data from the manufacturers, as well as data from the literature, when needed and justified.

In each estimate, the IQC material will contemplate the average results of each month's quality control results, regarding the year to which it refers, using two levels of control, normal/physiologic and pathologic levels, for each of the equipment in assessment. The data from the EQAS evaluation, performed monthly at the laboratory, provides for the bias results for each month, with the average corresponding to laboratory bias used in the subsequent calculations.

The pre-analytical component was based only on reports in the literature. Were considered different publications [79, 84-87] and was used the data for pre-analytical uncertainties from the studies of Fuentes-Arderiu, *et al.* [85], estimated regarding the global procedure variation, without differentiation of the individual steps.

The values assigned to the calibrators, as stated, were obtained from the manufacturer's specifications, in the case from the data on *Traceability and Uncertainty* - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche® (Appendix F), which presents values for Expanded Uncertainty (k = 2); that were assumed by the manufacturer to have been calculated in accordance with the "Guide to the expression of uncertainty in measurement" (GUM).

5.2.3 Expectations

By comparing the outcomes from the application of the two different uncertainty budgets, it was expected to proceed with the desired evaluation on the suitability of the different models to the clinical laboratory. Possibly this would enable the identification of eventual flaws in the estimates. It would also allow considering about the sources/components of uncertainty assessed, and its significant value, in way to conclude on the applicability and even validity of each of the models in study.

The results of this study were presented in the 20th IFCC - EFLM European Congress of Clinical Chemistry and Laboratory Medicine; 21th International Congress of Clinical Chemistry and Laboratory Medicine; 45th Congress of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC); in Milan (2013). The work was presented as a poster communication titled "*Measurement Uncertainty in the Medical Laboratory - Implementation and Evaluation of two Different Formulas in Clinical Chemistry Parameters: Total Cholesterol, Creatinine and Glucose Measurements*". (Appendix A)

5.3 Pre-analytical Uncertainty

The pre-analytical phase, although widely recognized as the stage comprising the largest percentage of error rates in the total testing process, estimated to account for 60% to 70% of all the errors and occurrences in the medical laboratory [88-91], it is also often taken for negligible as a source of variation, or uncertainty, to the final result, as long as its procedures are standardized.

However, these assumptions have already been disproved, or at least challenged, by several different authors [79, 84-87]. Despite not being considered by some measurement uncertainty approaches, that although recognizing the effects of the pre-analytical variation focus their attention only on the analytical phase, there are many who support its inclusion, defending that the variation associated to the pre-analytical phase should not be considered negligible and its value should be included when estimating measurement uncertainty in the medical laboratory [32, 59-61, 75]. The European Diagnostic Manufacturers Association (EDMA) supported these considerations in a position paper regarding the estimation of measurement uncertainties medical laboratories, when referring to the uncertainty sources to consider [92]. Also the Eurachem/Citac Guide for Uncertainty Quantification in Analytical Measurement [4] as well as the European guidelines on the expression of uncertainty in quantitative testing [3], although not explicitly covering the pre-analytical procedures, mention sampling and sample pre-treatment as a source of uncertainty.

When being considered as a source of uncertainty, the values of pre-analytical variation should be estimated by each laboratory, for its own conditions [92]. Ultimately, as addressed above, despite the standardization of the process, influences regarding phlebotomy and blood withdrawal; sample handling, including the pre-treatment process and processing time; the laboratory's conditions (such as the waiting room, the collecting area or the Individual booths) and materials (collection tubes, centrifuge and centrifugation

specifications), or samples transportation and storage; may alter the measurable amount of an analyte in a sample and end up adding variation to the final result. This pre-analytical variation should then be acknowledged and taken into account in an uncertainty budget. It should also be considered specific to each laboratory in a reasoning eventually similar to the one applied to the variations in the analytical phase, which are properly valued and quantified, in spite of all the efforts, guidelines and procedures towards standardization of methods, equipment and materials.

On this matter, being the literature extensive regarding the pre-analytical phase and errors, the bibliographical sources that provide quantitative information are rare, are not available for all tests/measurand and can hardly be considered wide-ranging or standardized so it can be directly and unconditionally applied in any other laboratory and on its results. This literature can and should be used in an initial stage of the measurement uncertainty estimation process, being afterwards replaced by the laboratory's own estimates. A laboratory's estimate means a direct and representative value of the laboratory's procedures and conditions, and so of its own pre-analytical uncertainty.

5.3.1 Gnóstica's Pre-analytical Uncertainty Study

Bearing in mind the presented facts, regarding the potential influence and significance of the pre-analytical phase in the total testing process, it was mandatory to include in this work a study of this uncertainty component/category and the quantification of the pre-analytical variation associated to the specific analytes in question.

Considering that each laboratory should then estimate its own pre-analytical uncertainty values, for any quantities tested, it was implemented a protocol to this purpose, aiming to quantify this variable to subsequently substitute the data previously considered from the literature by values that correspond directly to the laboratory's procedures and that are representative of its practices and associated variation.

With the approval of the Management and Technical Boards of "*Gnóstica - Medical Laboratory*", was developed and implemented an internal study to estimate and characterize the influence and variability from pre-analytical sources, associated to the laboratory's patient results in the analytes of Creatinine, Glucose and Total-Cholesterol (*The results of this study are not yet published, but a paper is in preparation to submit for publication in the Journal Clinical Chemistry and Laboratory Medicine* (CCLM)).

Thus, with the objective of estimate the uncertainty related to pre-analytical phase, were considered as sources of pre-analytical variation the following: sample collection (phlebotomy and handling); sample pre-treatment phase (processing and clotting time, before centrifugation); sample storage (refrigeration and freezing, after serum separation in different aliquots); sample transportation (considering the possible transportation of the samples by car to a central laboratory, after collection).

Gnóstica Laboratory, being NP EN ISO 15189 Accredited, has its procedures for the collection and for the handling of diagnostic blood specimen/samples by venipuncture standardized and validated. These standardized procedures were followed and the blood collection was performed by certified and trained phlebotomists, from the laboratory's technical staff. After signing an informed consent, 37 random users, attending the laboratory with programed routine tests previously ordered by their physicians, were voluntarily recruited to the study. The blood collection was made by the same phlebotomist, in both arms, on two different venous punctures, using the laboratory's standard collection tubes, with clot activator and gel for serum separation. For each person the samples were taken consecutively, reducing at the most the time interval between withdrawals, so that the effect of intra-individual variation could be as minimum possible, allowing it to be neglected.

Following the laboratory's standardized procedure all the tubes were centrifuged in a refrigerated centrifuge (4˚C), at 4500rpm for 15 minutes (Heraeus Megafuge 1.0R Refrigerated Benchtop Centrifuge).

The tests were performed on two identical *Cobas*® 6000 – c501 module (Roche®), operating in the same room, under the same conditions, handled by the same technicians and using the same lot numbers of reagents, control and calibration material (Cobas® c501 / c502 / c311 / c701 / c702 - C.a.s.f.), from Roche® (Appendix EF).

In all the tests the measurand for each analyte was the amount-of-substance concentration in the human serum samples analysed. For the Creatinine measurement the *Cobas*® 6000 – c501 module uses the Kinetic Jaffé Method (Rate blanked & compensated, Gen.2), a kinetic colorimetric assay. In alkaline solution, creatinine forms a yellow orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the sample. The assay uses "rate-blanking" to minimize interferences, namely by bilirubin. Glucose was performed by the Hexokinase/G-6-PDH Method, in which

hexokinase (HK) catalyses the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. Following this reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyse oxidation of glucose-6-phosphate by NADP to form NADPH. The concentration of the NADPH formed is directly proportional to glucose's concentration in the sample. Total Cholesterol was determined by CHOD/PAP Gen. 2 Method where cholesterol esters are hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and H2O2. In presence of peroxidase (POD), the formed hydrogen peroxide affects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye. The intensity of the color produced is directly proportional to cholesterol concentration. Roche's equipment methods and calibrators assure the traceability of the measurement to isotope dilution mass spectrometry (IDMS) reference measurement procedure.

For each individual, *Sample A* (obtained from tube 1), corresponded to the sample collected within the referred programed tests, being its results included as the reference result for *Sample B*. From the second puncture, always performed on the other arm, three tubes were withdrawn (tubes 2, 3 and 4), which provided Samples B, B1, B2, C and D, respectively, being B, B1 and B2 different aliquots from tube 2. The results from sample B were then used as the reference for the following samples. The study's design is schematically represented in Figure 9, below:

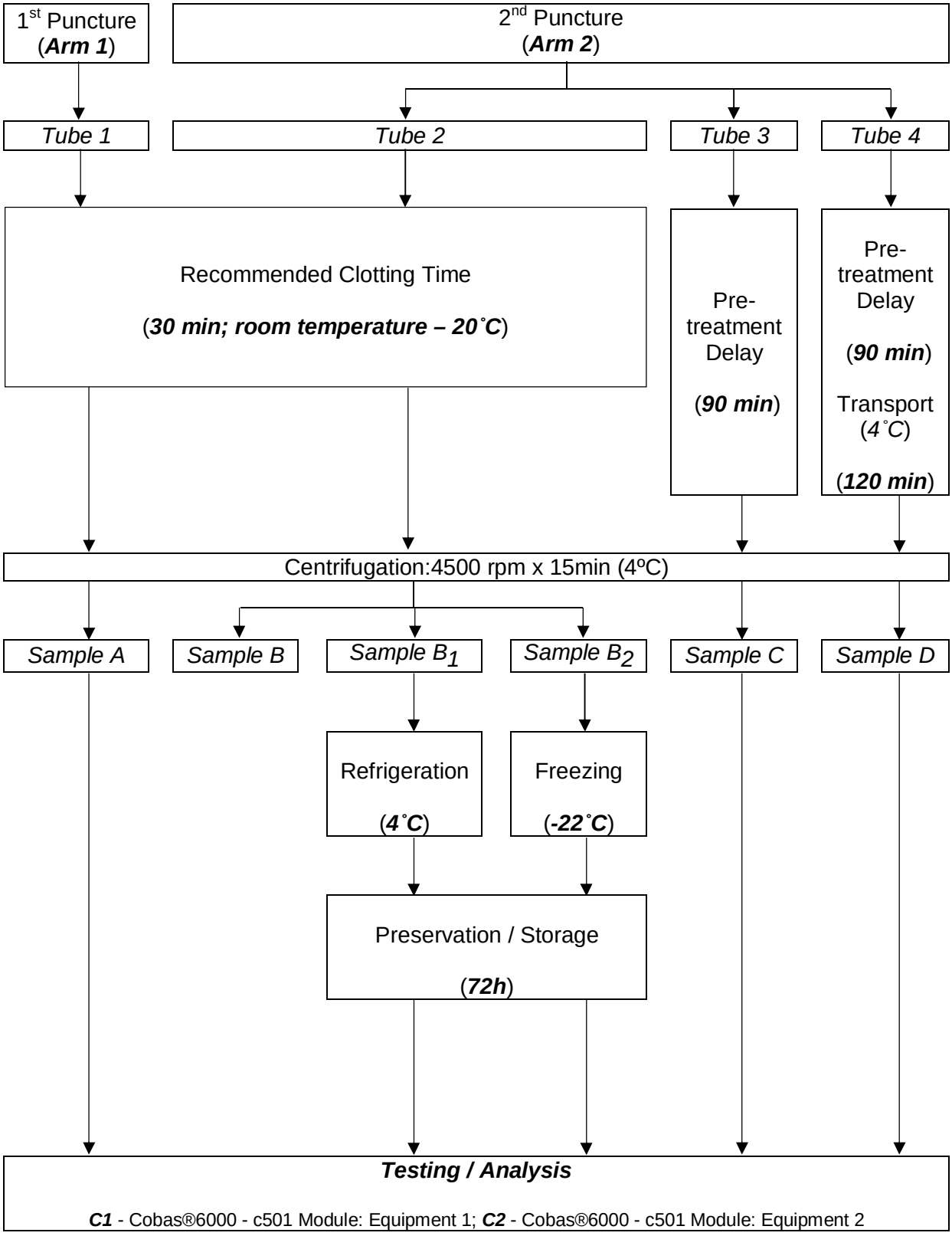


Figure 9 - Experimental design to estimate the uncertainty of the Pre-Analytical Phase.

Thus, after the 30 minutes of recommended clotting time, the Tubes 1 and 2 were centrifuged. From tube 2 three aliquots were separated, corresponding to samples B, B1 and B2. Samples A and B were analysed without delay and samples B1 and B2 were respectively refrigerated at 4˚C and frozen at -22˚C for 72h, before analysis. Tubes 3 and 4 were kept in the sample sorting area, at room temperature (20˚C) for 90 minutes, following then the procedure designated for each experiment. Tube 3 was centrifuged in the same conditions as the previous and then analysed. Tube 4 was transported by car, in a thermal transport case for diagnostic samples, with controlled temperature, for approximately 120 minutes and only then was centrifuged and analysed as the remaining tubes.

5.3.2 Determination of Gnóstica's Pre-analytical Uncertainty

So, the uncertainty related to sample collection and handling was characterised by the variations between the results from samples A and B. Then, comparing the results form sample B with samples B1, B2, C and D, allowed evaluating respectively the effects of sample storage (refrigeration and freezing), delay in the pre-treatment phase and regional transportation, and to estimate the standard uncertainty associated to each of these variability sources.

As stated before, according the definitions [2, 4, 35], the different sources of uncertainty that are combined into to a measurement uncertainty estimate, represent the several uncertainty components in consideration and are designated as standard uncertainties or relative standard uncertainties (u). Being, in general, the result of a measurement, they are usually given in the form of a standard deviation, a relative standard deviation (SD), or a coefficient of variance (CV %), as most commonly expressed in the medical laboratory, particularly in clinical biochemistry.

To obtain the pre-analytical uncertainties associated to each variable in study, the paired results between the reference samples and the different alternative/experimental procedures were evaluated. For such, each uncertainty source was separately studied, by applying a methodology previously used in studies of similar design [85, 87, 93-95].

Thus, the general coefficients of variation (CV %; for B, B1, B2, C and D), derived from the variances of each uncertainty source, were calculated with the mean of the 74 measurements done for each quantity in the correspondent evaluation (which includes analytical variation plus pre-analytical variation). For each quantity and variable in study, general analytical variance was derived from the difference between the results obtained for the different runs/analysis, by applying equation (5-5):

$$s^2 = \frac{\sum(x-y)^2}{2n} \quad \textbf{(5-5)}$$

Where, s^2 is the general analytical variance (analytical variance plus pre-analytical variance), x and y are the measurement results obtained for the different samples from the same individual (reference and in-study) and n is the total number of individuals.

Afterwards, the coefficients of variation regarding the pre-analytical variation (CV_{Pre} %) were calculated for each quantity by subtracting the CV_A, obtained from the day-to-day imprecision data from the laboratory's QMS, from the general CV calculated for each variable (B, B1, B2, C and D), which resulted in each variability source's standard uncertainty (u).

Finally, using equation (2-1), the standard uncertainties were combined ($u_{c\ pre}$) and, to conclude, the Expanded Pre-analytical Uncertainty (U_{pre}) was obtained applying equation (5-3), on page 55, with k=2, for a coverage probability of 95%.

5.4 Measurement Uncertainty in the Medical Laboratory (II)

5.4.1 Application of the different MU estimate formulae - Gnóstica´s Pre-analytical Uncertainty (*2013 Update*)

This step consisted in combining the protocols above, in sections 5.2 and 5.3, updating the values of the several components, or uncertainty sources, with new data (*year: 2013*). The results regarding the pre-analytical uncertainty component obtained in the previous point were introduced on equation (5-2), on page 54, replacing the data that was being used from the literature, enabling the determination of MU with the laboratory's own pre-analytical variability estimate.

5.5 IPAC's Accreditation Auditing

In Portugal, the laboratories accredited by the ISO 15189 Standard had the first preliminary auditing under the new requirements of the ISO 15189:2012 guidelines in 2015, which then reflected the implementation of the Portuguese version, *NP EN ISO 15189:2014*.

To this purpose, the first results of this study and collaboration, regarding the tests already addressed, Creatinine, Glucose and Total Cholesterol, were presented to the auditing team, for IPAC's consideration, as an initial approach for compliance with these new requirements. These were validated, contributing for the renewal of the accreditation certificate (Appendix G).

Later on, early this year (2016), on a follow up auditing, having the previous report been accepted and positively considered, the laboratory was required to present evidence for the remaining 93 tests under the scope of the accreditation, as well as for the methodology applied in the final MU estimates.

5.6 Measurement Uncertainty in the Medical Laboratory (III)

Following the previous studies, and after the first practical test on the applicability of the formulae, it was decided the final approach to implement as Gnóstica's model to the MU estimates. The analysis and reflection regarding the results obtained until now, were the in the basis of the decisions made before getting to the final equation. This third application of the equation, still considering a last adjustment, or update, .will concentrate only on the chosen formula, being applied to the year's 2015 data, once again collecting information form the QMS, from the pre-analytical study and from the manufacturer.

5.6.1 Gnóstica's MU Estimate - Final Formula (MU-B)

This study, being an applied research project focused in a single laboratory, was developed with the purpose of reaching a formula able to fulfil and respond to the laboratory's necessities regarding the accreditation requirements and procedures. Also, it was intended to meet Gnóstica's own will and determination of offering a service, measurement methods and results with proven and recognized quality, complying with the international quality standards, by using, or including in the process, the data available from its Quality Management System.

Thus, the provisional results obtained in sections 6.2 and 6.4, as well as the outcomes and experience from section 6.5 results external assessment, were considered and discussed along with the Laboratory's Technical Board. The

initial approaches and its outcomes were reviewed and it was finally achieved a formula that fulfilled that primary objective.

The way forward regarding the final approach and equation which to be implemented, was also decided weighting over previous published work on the subject, being that guidance based on publications of National Accreditation Bodies [96, 97], on reference documents like the NordTest Report [35], or paper articles and studies from different authors [31, 37, 58, 59, 61, 76, 82] on the estimate of measurement uncertainties in the medical laboratory, as done before. However, having considered this different information, the final formula was defined mostly aiming to reach a tailored approach, found to be suitable to the laboratory's intents, conditions and available data, that was simultaneously fit for purpose and that complied with the identified requirements.

Thus, was decided that the final formula should include the uncertainty values assigned to the calibrators, bearing in mind that these data, when available, should be used in the MU estimate, adding the traceability component to the estimate and data regarding the method's trueness, over the bias values. These last, when identified should be eliminated or minimised, not being accounted for in the MU [33, 73], as long as proper traceability has been assured.

Recently, Farrance, *et al.* affirmed that, whenever a stable performance is assured, with minimal deviation, confirmed over internal traceability studies or proficiency testing surveys, assuring that it remains internally consistent and consistent with the comparator, "bias becomes largely irrelevant". This considered bearing in mind that "result interpretation is largely by comparison", being the test results always compared to patient's previous results or population reference intervals [98].

Nevertheless, bias might be considered significant. Whenever calibrator and method's trueness and traceability cannot be assured or when, on its assessment, it violates the minimum desirable specifications, being considered international performance tables or a group's mean in proficiency testing, it clearly indicates the presence of undesirable bias values. In these situations, if

not corrected and if considered significant after proper evaluation [25, 35, 99], it could be combined in the estimate.

Other defended point of view stands that treating bias as a variance and adding it to the MU estimate can lead to misjudgements of its influence on the test's performance, considering that it should never be included in the MU, but assessed and reported separately [100]. Once again, Farrance, *et al.*, states that "having separate procedures for the assessment of imprecision and bias however, is simple and inherently logical" and that for a laboratory "what is required by ISO 15189 is to document (and understand) your chosen process" [98].

The influence of the pre-analytical phase on laboratory's measurements and results was already addressed in section 5.3 and is demonstrated by the results on section 6.3. Although always referred to in guidelines and in different studies or opinion articles, this uncertainty source is frequently disregarded in the final uncertainty estimates. So, assuming the existing variations, even in standardized systems, was decided to also include this component in the final estimate.

Finally, taking into to account the necessity of obtaining a flexible and broad model, covering the range of physiological and pathological values of test results, it was decided to calculate an overall long term value for the random variation, or laboratory's intermediate precision component, by the use of a global and inclusive CV (CV_{pooled}), being it a weighted mean of a large time interval (one year) at different concentration levels. This methodology recently proposed [99, 101] in other "*top-down*" approach studies, based on the NordTest Report TR537 [35], had already been defended to be the best to combined different imprecision estimates, for example for different levels of an IQC for the same measurement method [102]. The proposed methodology presents a random component of uncertainty calculated using the weighted mean of the monthly CV, of at least 6 months. Thus, in this study, using data from IQC performed at normal and pathological concentration levels, the long

term imprecisions (one year period) were combined with base on equation (5-6):

$$u_{Imprecision} = \sqrt{\frac{\left((n_A - 1) \times {CV_A}^2\right) + \left((n_B - 1) \times {CV_B}^2\right)}{\left((n_A + n_B) - 2\right)}} \quad \textbf{(5-6)}$$

In which:

- ***A)*** *is the Level 1, or Normal Level Control:*
- ***B)*** *is the Level 2, or Pathological Level Control;*
- ***n)*** *represents the number of entries/months considered for the pooled CV calculation;*
- ***CV)*** *is the mean coefficient of variation for that period.*

Thus, with the above update in its application, the final MU-B model formula, initially represented in equation (5-2), on page 54, was then considered to be the one best fitting to the laboratory's objectives and to the study's purposes. This final equation and its outcomes were considered to be representative of the measurement process and the best estimate for the uncertainty of measurement and of the results. Thereby, it was lastly applied to the laboratory's data, for a final assessment.

5.6.2 Standard Uncertainties' Index

Aiming to provide a visualization of the relative contributions from the different relative standard uncertainties was calculated the index for each of the variation sources, within the combined uncertainty for the analytes and measurements in study.This exercise brings the benefit of easy and direct identification of the weight of every component in the global estimate. This allows for the laboratory staff, usually the quality department and management, to analyse the outcomes and, whenever necessary or justified, prepare any eventually improvement actions.

5.7 MU Estimate Final Tool – Gnóstica's working spreadsheet

The final step, and objective of this work, was to develop a practical tool that could be easily implemented in the laboratory's quality routine procedures, for the estimation of measurement uncertainty of its results.

The best way to accomplish that was to create an Excel® (*Microsoft*) spreadsheet, that contemplate the uncertainty sources considered and that, by simply updating the monthly or annual values form the laboratory's QMS and other documentation considered, would able to deliver the Laboratory's Measurement Uncertainty for the selected tests or measurements.

It was also given room for some improvement opportunities to the formula, with the possibility of introducing other variable as uncertainty sources, considering for example specific studies or measurement conditions, or for specific processes of monitoring patient's health and diseases.

6 RESULTS

The following chapter contains the main results obtained in this project. These results were collected over the period from 2011 to 2016, from Gnóstica – Medical Laboratory.

Data collection consisted in three different dimensions. First, for the retrospective study aiming to present the quality theme and to characterize the laboratory was gathered data from years 2003 to 2010 (section 6.1). Then for the uncertainty measurement project itself, the data was collected in 2012, 2014 and 2016, respectively for sections 6.2, 6.4 and 6.5. As for the third, regarding the practical laboratorial work and data collection, for section 6.3, was developed between 2012 and 2013, with the data being obtained directly from laboratorial results reports. All the remaining data was obtained from the annual quality reports, compiled by the Quality Management Department of Gnóstica, within the procedures of the implemented Quality Management System.

6.1 Quality Indicators

This Section presents in Table 6-1 a summarized description of the laboratory's overall performance characteristics, general quality data and the quality perception, as perceived by its users. The information assembled by the laboratory's Quality Management System, allows characterizing the laboratory and its quality practices, by presenting the improvements and evolution resulting from the implementation of planned quality policies, through the outcomes of the registered quality indicators.

The data was accessed and gathered from the laboratory's quality reports that assemble information from the anonymous questionnaires, which are applied every year to its users, assessing user's satisfaction and their perception regarding the service's quality; and from the quality system records, which registers and follows the occurrences and non-conformities of the daily routine, with indicators that cover the different phases of the total testing process, from the pre-analytical, starting with the user and analysis registry, to the post-analytical, until the delivery of the results.

In respect of to the presented indicators, the "*User's Overall Satisfaction*" is measured directly from the users' replies to the annual survey, with the results being graphically represented in the laboratory's quality reports. These are shown, as examples, in the Appendix H, regarding the years 2005-2007, 2009 and 2010. The graphical representation of the "*Waiting Time %*", which reveals the user's satisfaction and evaluation concerning the time expended when attending the laboratory for the necessary procedures to the scheduled medical analysis (blood withdrawal or other biological sample collection), is also presented in Appendix I, showing examples for the years 2003-2007.

To both of these categories the response options in the questionnaires are: *very good, good, sufficient, mediocre* and *bad*, being here presented the most demonstrative categories, which represent always around 95% or more of the grand total.

As regards to the remaining indicators, this data is monitored by computer record throughout the year, covering the different phases of the laboratorial procedures, as mentioned. Here, in the table below, are presented:

- *Registry Errors (%)*: representing the percentage of detected errors that lead to posterior changes in the registry, being that the most common are regarding the user's name/identification or are analytes/tests related, involving exchanged tests (with added or missing tests) ;

- *Sample Collecting Repetition (%)*: indicating the percentage of sample collection repetitions derived from errors detected in pre-analytical sample screening;

- *Waiting Time (minutes)*: which shows the data from the computerized records concerning the time spent by users when attending the laboratory, between registry and sample collection;

- *Repeat Testing (#)* and *Repeat Testing (%)*: being the number (#) and respective percentage of tests/analysis repeated, in view of the total number of tests/analysis executed in that same year;

- *EQAS*: this category presents the total number of *Schemes (#)* in which the laboratory participates, with the indication of the average percentage of *Correct Results (%)*, in those same schemes, per year.

- *Fulfilment of Delivery Date (%)* and *Same Day results (%)*: reveals the percentage of results delivered on the predicted deadline and of results delivered on the day of the sample collection;

- *Non-Conformities (NC; #)* and *Non-Conformities (NC; %)*: a non-conformity (NC) indicates an error detected after the emission/delivery of a patient's result that implies the repetition of any procedure and involves re-calling that user to the laboratory. Here it is presented the number of NCs (#) and its respective percentage, in view of the total of number users/patients of the respective year.

- *NC - Reception/Registry (#)* and *NC - Reception/Registry (%)*:represent the number (#) and percentage (%) of NCs with particular respect to the Reception/Registry procedures. Where, again, the most common error causes occur in the user's name and personal identification data registration process or are analytes/tests related (exchanged, added or missing tests) during that same registry.

The last data presented are the annual totals for the number of Completed Questionnaires, Users/Patients registered and attended at the laboratory, as well as Tests/Analysis performed by the Laboratory.

Table 6-1 – Gnóstica - Medical Laboratory: Evolution of the quality indicators (2003 - 2010)

Quality Indicators			2003	2004	2005	2006	2007	2008	2009	2010
User's Overall Satisfaction (%)	Very Good		*44,0*	*59,9*	*63,7*	*45,2*	*44,1*	*57,9*	*49,6*	*45,9*
	Good		*45,7*	*36,1*	*33,0*	*48,2*	*50,9*	*35,3*	*41,7*	*50,0*
	Sufficient		*3,1*	*1,3*	*1,1*	*4,4*	*4,5*	*2,1*	*3,1*	*1,5*
Pre-Analytical Phase	Registry Errors (%)		*2,8*	*2,8*	*3,1*	*4,1*	*3,0*	*3,5*	*2,8*	*4,8*
	Sample Collecting Repetition (%)		*1,2*	*1,1*	*1,1*	*1,1*	*1,2*	*1,1*	*0,9*	*0,7*
	Waiting Time (%)	*Very Good*	27,0	35,5	30,3	27,0	28,0	33,9	34,9	31,9
		Good	42,0	44,1	54,3	43,6	54,5	55,8	47,5	48,6
		Sufficient	26,0	19,5	15,4	23,9	15,9	19,6	16,9	16,6
	Waiting Time (minutes)		---	---	21,2	25,4	15,5	12,1	11,9	9,7
Analytical Phase	Repeat Testing (#)		20157	6473	7294	9758	7758	12524	11814	8180
	Repeat Testing (%)		*6,10*	*1,70*	*1,90*	*2,50*	*1,60*	*2,17*	*1,98*	*1,01*
	EQAS	Tests (#)	20	22	21	22	24	28	28	29
		Correct Results (%)	*90*	*90*	*94*	*94*	*96*	*92*	*95*	*96*

Table 6 – 1: Gnóstica - Medical Laboratory: Evolution of the quality indicators (2003 - 2010) – Cont.

Quality Indicators		2003	2004	2005	2006	2007	2008	2009	2010
Post-Analytical Phase	Fulfilment of Delivery Date (%)	93	94	94	93	94	91	88	95
	Same day results (%)	52	54	57	52	39	36	30	52
	Non-Conformities (NC; #) – Total	45	68	44	71	53	97	38	25
	Non-Conformities (NC;%) – Total	*0,12*	*0,17*	*0,11*	*0,17*	*0,11*	*0,18*	*0,06*	*0,03*
	NC - Reception/Registry (#)	22	48	36	64	39	77	30	20
	NC - Reception/Registry (%)	*48,9*	*70,6*	*81,8*	*90,1*	*73,6*	*79,4*	*78,9*	*80,0*
Completed Surveys (#)		**293**	**227**	**179**	**166**	**427**	**235**	**710**	**1240**
Users / Patients (#)		**36640**	**40790**	**40984**	**42129**	**49553**	**55269**	**59005**	**84327**
Tests /Analysis (#)		**330441**	**380789**	**383911**	**390301**	**484867**	**577151**	**596655**	**810432**

NC; # - Total number of detected/registered Non-Conformities; ***NC; %*** - Percentage, considering the total number of users/patients.

6.2 Measurement Uncertainty in the Medical Laboratory

Having defined the approaches and formulae to apply in the first practical incursion in estimating the measurement uncertainty at Gnóstica Laboratory, the data regarding the different sources of results variation being considered for the MU estimates was then assembled.

This section's tables show the data gathered from the Laboratory's QMS, which provided the laboratory's results for imprecision and bias, taken from the IQC data and from the EQAS results.

Additionally, are presented the data from the manufacturers' specifications, with regards to the calibrators' uncertainty information, and from the literature in the case of the pre-analytical uncertainty data.

These information and data for the several uncertainty components was distributed into different tables, by grouping on the first subsection the random variability source, on the next the systematic variability, both from Gnóstica's internal quality system data. To these presented data is added, on sub-section 6.2.3, the calibrator's and the pre-analytical uncertainty values, as a complete resume of all the variability sources entering the estimate.

Lastly, also on sub-section 6.2.3, are presented the primary results from the application of the two different formulae for the MU estimate to the data collected, as well the results of the determination of the Total Error, for that same period, for comparison.

On note regarding the units displayed for the analytes and different parameters presented, despite metrological traceability being preferably reported to SI units, with the use of the amount of substance (mole) unit for expressing laboratory results, the data is presented using traditional units (mg/dL), as these are the units adopted and used by the laboratory on its reports. Being embedded on the Portuguese healthcare system, Gnóstica uses the units most commonly applied in this reality, which were kept in the study.

To complement this information, this practice can perhaps be better understood when considering the results of a recent study by Ceriotti, *et al.*, on behalf of the EFLM WG-H (Working Group on Harmonisation of total testing process), on "Harmonization initiatives in Europe". In the study, when addressing the current use of SI units in Europe, in a universe of 40 countries surveyed, Portugal stands in the low bottom, with 10-20% of utilization. Being the unit of measurement an important issue, the most problematic situation identified was for some few countries who declared that they are *not in favor* of changing or promoting the adoption of SI units, as it was the case of Portugal.

6.2.1 Random Variability: determination of the laboratory's intermediate precision

Regarding the year of 2011, are here presented the data from the IQC for the determination of the imprecision, giving the quantitative expression to the laboratory's intermediate precision, sometimes designated as within laboratory reproducibility or within-lab long term reproducibility. This represents an important source of variation in the medical laboratory and as such a fundamental component of the MU estimate and demonstrative of the random analytical variation of measurement's results.

For each of the analytes considered in the study, creatinine, glucose and total cholesterol, designations that represent the measurement of each substance's concentration in human serum samples, is presented a table, respectively Table 6-2, Table 6-3 and Table 6-4, introducing the IQC monthly mean values, and the final mean value for the year in question (*IQC* ($\overline{X}$)). Likewise, are also presented the coefficients of variation for each determination method, as well as the monthly mean values and the final annual mean, being this the value of the laboratory's total *Imprecision* (CV%), and the referred numerical expression of the laboratory's intermediate precision. These values are shown for both levels of the quality control material in use, *normal* and *pathological*, being considered the two identical equipment operating in the laboratory, the Cobas® 6000–c501 (Roche®) auto-analyser, identified as *C1* and *C2*.

Table 6-2 – Total Imprecision for the determination of Creatinine's concentration in human serum samples, by the Kinetic Jaffé Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	Medical Decision Value		Month	IQC (mg/dL) -Normal-	IQC (mg/dL) -Pathological-	IQC ($\bar{X}$) (mg/dL) -Normal-	IQC ($\bar{X}$) (mg/dL) -Pathological-	IQC (CV%) -Normal-	IQC (CV%) -Pathological-	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-
Creatinine	*1,2 mg/dL*	**C1**	*Jan*	1,07	3,74	1,10	3,78	5,4	4,0	***4,31***	***3,31***
			Feb	1,11	3,80			4,8	4,0		
			Mar	1,13	3,79			5,4	4,5		
			Apr	1,08	3,74			4,8	3,2		
			May	1,11	3,74			4,4	3,8		
			Jun	1,11	3,77			4,2	2,8		
			Jul	1,10	3,83			3,6	2,6		
			Aug	1,11	3,79			4,0	2,9		
			Sep	1,12	3,83			3,6	2,5		
			Oct	1,09	3,78			4,4	3,4		
			Nov	1,13	3,85			3,8	3,0		
			Dec	1,08	3,74			3,3	3,0		
		C2	*Jan*	1,11	3,86	1,12	3,82	4,9	3,9	***3,51***	***3,03***
			Feb	1,12	3,86			3,8	2,6		
			Mar	1,11	3,76			2,9	3,3		
			Apr	1,12	3,88			2,7	2,9		
			May	1,12	3,84			2,9	2,7		
			Jun	1,12	3,80			3,5	2,8		
			Jul	1,11	3,82			2,9	2,7		
			Aug	1,10	3,73			4,0	2,8		
			Sep	1,13	3,85			3,8	3,2		
			Oct	1,10	3,75			3,5	3,3		
			Nov	1,13	3,86			3,2	3,1		
			Dec	1,13	3,87			4,0	3,1		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

Table 6-3 – Total Imprecision for the determination of Glucose's concentration in human serum samples, by the Hexokinase Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	Medical Decision Value		Month	IQC (mg/dL) -Normal-	IQC (mg/dL) -Pathological-	IQC $(\overline{X})$ (mg/dL) -Normal-	IQC $(\overline{X})$ (mg/dL) -Pathological-	IQC (CV%) -Normal-	IQC (CV%) -Pathological-	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-
Glucose	110 mg/dL	**C1**	Jan	97,52	235,15	97,44	233,60	1,1	1,9	***1,36***	***1,36***
			Feb	97,86	234,83			1,7	1,4		
			Mar	97,52	232,52			1,5	1,7		
			Apr	96,68	233,44			1,0	1,4		
			May	97,58	233,40			1,4	0,9		
			Jun	98,73	236,23			1,1	1,0		
			Jul	97,95	234,20			1,5	1,5		
			Aug	96,69	231,28			1,4	1,3		
			Sep	96,96	232,37			1,8	1,5		
			Oct	96,48	232,07			1,4	1,7		
			Nov	97,64	234,34			1,2	1,1		
			Dec	97,66	233,41			1,2	0,9		
		C2	Jan	97,62	234,90	97,98	234,71	1,1	1,9	***1,63***	***1,75***
			Feb	98,12	235,97			1,2	1,1		
			Mar	97,80	232,67			1,6	1,7		
			Apr	97,23	234,20			1,3	1,3		
			May	97,88	233,68			1,5	1,6		
			Jun	99,70	238,57			1,1	1,0		
			Jul	98,10	234,88			2,1	1,9		
			Aug	97,88	233,13			2,0	2,1		
			Sep	97,96	233,65			1,9	1,9		
			Oct	97,00	234,19			2,0	2,5		
			Nov	99,40	237,79			1,6	1,5		
			Dec	97,07	232,95			2,1	2,5		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

Table 6-4 – Imprecision for the determination of Total Cholesterol's concentration in human serum samples, by the CHOD-PAP Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	Medical Decision Value		Month	IQC (mg/dL) -Normal-	IQC (mg/dL) -Pathological-	IQC ($\bar{X}$) (mg/dL) -Normal-	IQC ($\bar{X}$) (mg/dL) -Pathological-	IQC (CV%) -Normal-	IQC (CV%) -Pathological-	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-
Total Cholesterol	*190 mg/dl*	**C1**	*Jan*	94,23	179,16	95,76	180,06	2,2	2,0	***1,88***	***1,62***
			Feb	95,21	180,30			2,4	2,0		
			Mar	95,24	177,87			1,5	1,4		
			Apr	97,28	181,66			2,4	1,8		
			May	95,52	178,87			2,8	2,1		
			Jun	96,24	180,68			1,2	1,1		
			Jul	96,48	181,06			2,3	2,0		
			Aug	96,08	178,98			1,8	1,4		
			Sep	96,35	180,76			1,4	1,2		
			Oct	94,40	179,42			1,8	1,8		
			Nov	96,49	181,48			1,5	1,6		
			Dec	95,55	180,45			1,3	1,0		
		C2	*Jan*	94,56	180,02	95,94	180,45	2,8	3,2	***2,20***	***1,80***
			Feb	96,54	182,41			3,2	2,1		
			Mar	94,91	177,39			2,1	2,3		
			Apr	95,14	178,95			1,6	1,6		
			May	97,43	180,78			2,0	1,1		
			Jun	97,84	182,07			2,3	1,0		
			Jul	96,54	181,49			2,5	2,0		
			Aug	95,28	180,57			3,3	2,4		
			Sep	95,42	180,08			1,2	1,1		
			Oct	94,46	179,15			1,6	1,6		
			Nov	96,89	182,00			1,4	1,5		
			Dec	96,22	180,55			2,4	1,7		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

6.2.2 Systematic Variability: Trueness - Laboratory's Bias determination

Also for the year 2011 and still quantifying the performance characteristics of the laboratory's selected methods, the following tables present the laboratory's trueness quantitative expression, in the form of each method's bias, determined from the EQAS results. Once more with each table referring to a different measurand, Bias, defined as the difference between the result of the laboratory measurement and the accepted reference value for the EQA programme, is here expressed as a percentage, determined monthly by the equation (6-1), where x is the laboratory's result and y_0 the target or accepted reference value for the EQAS:

$$Bias\ (\%) = \frac{(x - y_0)}{y_0} \cdot 100 \qquad \textbf{(6-1)}$$

Where x represents the laboratory's result for the EQAS, y_0 is the EQAS Target Value for the assessment in question.

So, on Table 6-5, Table 6-6 and Table 6-7 can be observed the monthly values determined from the participation on EQAS, as well as the final numerical expression for the systematic variability of the measurement, in the last column, quantifying trueness as the laboratory's bias, with the year's mean value.

Table 6-5 - Trueness/Bias assessment for the determination of Creatinine's concentration in human serum samples, by the Kinetic Jaffé Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	*Medical Decision Value*	*Month*	*EQAS -Result-* (mg/dL)	*EQAS -Target-* (mg/dL)	*EQAS -Error-* (%)	*Trueness* (|BIAS| %)
Creatinine	*1,2 mg/dL*	*Jan*	0,60	0,67	-10,6	***3,85***
		Feb	1,41	1,52	-7,1	
		Mar	6,42	6,65	-3,4	
		Apr	1,32	1,37	-3,7	
		May	3,64	3,88	-6,2	
		Jun	4,02	4,10	-1,9	
		Jul	6,48	6,67	-2,8	
		Aug	1,39	1,52	-8,6	
		Sep	4,08	3,96	3,2	
		Oct	4,23	4,11	3,0	
		Nov	3,81	3,87	-1,6	
		Dec	1,29	1,38	-6,5	

Table 6-6 - Trueness/Bias assessment for the determination of Glucose's concentration in human serum samples, by the Hexokinase Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	*Medical Decision Value*	*Month*	*EQAS -Result-* (mg/dL)	*EQAS -Target-* (mg/dL)	*EQAS -Error-* (%)	*Trueness* (|BIAS| %)
Glucose	*110 mg/dL*	*Jan*	35,0	35,5	-1,4	***1,15***
		Feb	106,0	107,8	-1,7	
		Mar	345,0	348,5	-1,0	
		Apr	109,0	109,6	-0,6	
		May	257,0	259,9	-1,1	
		Jun	278,0	275,5	0,9	
		Jul	345,0	348,2	-0,9	
		Aug	106,0	107,5	-1,4	
		Sep	194,0	199,5	-2,7	
		Oct	270,0	275,3	-1,9	
		Nov	259,0	259,3	-0,1	
		Dec	107,0	109,0	-1,9	

Table 6-7 - Trueness/Bias estimate for the determination of Total Cholesterol's concentration in human serum samples, by the CHOD-PAP Method, on Cobas 6000 auto-analyser, module c501, from Roche®. (*Data regarding the year 2011*)

Test	*Medical Decision Value*	*Month*	*EQAS -Result-* (mg/dL)	*EQAS -Target-* (mg/dL)	*EQAS -Error-* (%)	*Trueness* (|BIAS| %)
Total Cholesterol	*190 mg/dl*	*Jan*	114,0	118,2	-3,6	***0,95***
		Feb	154,0	159,6	-3,5	
		Mar	303,0	303,1	0,0	
		Apr	152,0	156,2	-2,7	
		May	278,0	280,8	-1,0	
		Jun	287,0	292,2	-1,8	
		Jul	310,0	302,7	2,4	
		Aug	157,0	159,8	-1,7	
		Sep	208,0	211,9	-1,9	
		Oct	298,0	293,2	1,6	
		Nov	289,0	281,2	2,8	
		Dec	153,0	156,3	-2,1	

6.2.3 Implementation of the MU estimate formulae: *MU-Model A Formula and MU-Model B Formula*.

Finally, Table 6-8 gathers the previous presented data, on precision and trueness, for both equipment (*C1* and *C2*) and all three analytes, adding the calibrator's and the pre-analytical uncertainty values, thus completing the necessary data from of all the variability sources being considered and contributing with a partial value to the final MU estimate.

The uncertainty declared by the manufacturer for the calibrator assigned value corresponds to the expanded uncertainty (with a coverage factor k = 2, giving a level of confidence of approximately 95%). This value must be divided by its coverage factor, giving the standard uncertainty associated to the calibrator, and then by the assigned value to obtain the relative standard uncertainty (CV%). As for the pre-analytical uncertainty values, are given in the literature as relative standard uncertainties, allowing its direct application.

The estimates are then presented on Table 6-9, where can be observed the values determined from the application of the designated formulae to Gnóstica's quality assessment data and to the remaining collected data, all included in the estimating, under the defined criteria, as relative uncertainties. Those calculations' results are presented for Total Error, as previously mentioned, but more importantly for the MU-A formula, that restrictedly used the laboratory's IQC data and EQAS results, for imprecision and bias, and for the MU-B formula, which considered the data associated to the calibrating materials and the pre-analytical variation as uncertainty sources. Thus, after the relative uncertainties being combined, the results were multiplied by a coverage factor (k=2, for a coverage probability of 95%), to give the final Expanded Uncertainty (U). The last two columns, on the right, give information about the quality performance specifications and goals, for matters of enabling comparing the different equations' outcomes.

Table 6-8 – Data collection (Imprecision, Bias, Calibrator Uncertainty and Pre-analytical Uncertainty) for the implementation of the different MU estimating Formulae in study *(Data regarding the year 2011)*

Test		***Imprecision (CV%)*** *-Normal-*	***Imprecision (CV%)*** *-Pathological-*	***Trueness (|BIAS| %)***	***Calibrator Expanded Uncertainty*** (mg/dL) [a]	***Calibrator Value*** (mg/dL)	***Calibrator Standard Uncertainty*** (CV %)	***Pre-analytical Uncertainty*** (CV %) [b]
Creatinine	***C1***	***4,31***	***3,31***	***3,85***	***0,06***	***4,1***	***0,71***	***2,3***
	C2	***3,51***	***3,03***					
Glucose	***C1***	***1,36***	***1,36***	***1,15***	***1,63***	***193***	***0,42***	***3,2***
	C2	***1,63***	***1,75***					
Total Cholesterol	***C1***	***1,88***	***1,62***	***0,95***	***1,46***	***165***	***0,44***	***1,2***
	C2	***2,20***	***1,80***					

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2
[a] – Traceability and Uncertainty - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche®
[b] – *Pre-metrological (Pre-analytical) Variation of Some Biochemical Quantities*. Fuentes-Arderiu, *et al.* [85]

Table 6-9 – Estimated values for Total Error, MU Model-A and MU Model-B Formulae *(Data regarding the year 2011)*

Test		*Total Error -Normal- (%)*	*Total Error -Pathological- (%)*	*MU (A) -Normal- (%)*	*MU (A) -Pathological- (%)-*	*MU (B) -Normal- (%)*	*MU (B) -Pathological- (%)*	*TEa (%) European Table [17]*	*TEa (%) CLIA Table [18]*
Creatinine	***C1***	***12,5***	***10,5***	***11,6***	***10,2***	***9,9***	***8,2***	**8,9**	**15**
	C2	***10,9***	***9,9***	***10,4***	***9,8***	***8,5***	***7,7***		
Glucose	***C1***	***3,9***	***3,9***	***3,6***	***3,6***	***7,0***	***7,0***	**6,9**	**10**
	C2	***4,4***	***4,6***	***4,0***	***4,2***	***7,2***	***7,3***		
Total Cholesterol	***C1***	***4,7***	***4,2***	***4,2***	***3,7***	***4,6***	***4,1***	**8,5**	**10**
	C2	***5,3***	***4,5***	***4,8***	***4,1***	***5,1***	***4,4***		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2.

6.3 Pre-analytical Uncertainty

The pre-analytical phase, comprising the largest percentage of error rates in the total testing procedures, has already been proved to be an effective source of variation potentially affecting the medical laboratory test results. Consequently, several authors have stated that the variation associated to the pre-analytical phase should not be considered negligible and its value should be included when estimating measurement uncertainty in the medical laboratory

On this particular subject, and source of uncertainty, the references and data from the literature can and should be used in an initial stage of the implementation of measurement uncertainty estimation process in a laboratory.

After that period, it should be replaced by the laboratory's own estimates. A laboratory's estimate means a direct measurement applied to the laboratory's procedures and conditions, being a specific and representative value of its own pre-analytical uncertainty.

6.3.1 Gnóstica's Pre-analytical Uncertainty Study

Recognising the added value of estimating and knowing Gnóstica's own pre-analytical uncertainty values, was implemented a protocol to this purpose, aiming to quantify this variable associated to the analytes in study. The objectives were to confirm the findings in other studies and to, subsequently, be able to substitute the data previously considered from the literature, when estimating the laboratory's MU, by values that corresponded directly to the laboratory's procedures and that were representative of its practices and its specific variability.

With the approval of the Management and Technical Boards of "Gnóstica - Medical Laboratory", was developed and implemented an internal study, based on the laboratorial determinations of the concentration of the analytes Creatinine, Glucose and Total-Cholesterol, in its patient serum samples. Thereby, being NP EN ISO 15189 Accredited Laboratory, Gnóstica has its procedures for the collection and handling of diagnostic blood standardized and validated. These procedures were followed and the study was realized using the laboratory's own materials and according to its day-to-day conditions, was performed by its phlebotomists/laboratory technicians and the tests executed during the daily routine work, with the laboratory's routine equipment, the Cobas® 6000 – c501 module (Roche®).

In the study here considered as sources of pre-analytical variation the following: sample collection (phlebotomy and handling); sample pre-treatment phase (processing and clotting time, before centrifugation); sample storage (refrigeration and freezing, after serum separation in different aliquots); sample transportation (considering the possible transportation of the samples by car to a central laboratory, after collection). The protocol was previously described in Section 5.3.1 and is represented schematically in Figure 9.

Table 6-10, Table 6-11 and Table 6-12 shows the obtained results for the 37 individuals, respectively for the three different analytes studied, Creatinine, Glucose and Total-Cholesterol, for the different samples considered from these volunteer participants, randomly selected from the laboratory's users, according to the study planning

On the referred results tables, the different samples from every individual are identified, considering the variation source that each would allow to assess, as follows:

A) Reference measurement sample

B) Study of the uncertainty associated to the sample collection and handling;

B1) Evaluation of the effects of sample storage (refrigerated samples);

B2) Evaluation of the effects of sample storage (frozen samples);

C) Evaluation of the effects of sample processing (delay in the pre-treatment);

D) Evaluation of the effects of regional transportation.

Table 6-10 – Creatinine measurement: Kinetic Jaffé Method

(Sample analyte data / mg/dL)

Samples	*A*	*B*	*B1*	*B2*	*C*	*D*
1	1,58	1,58	1,63	1,62	1,65	1,57
2	0,56	0,64	0,58	0,58	0,57	0,58
3	0,73	0,75	0,72	0,76	0,72	0,72
4	0,75	0,72	0,72	0,73	0,69	0,65
5	0,68	0,67	0,67	0,70	0,69	0,68
6	0,94	0,82	0,85	0,88	0,84	0,88
7	0,64	0,62	0,66	0,66	0,63	0,63
8	0,73	0,73	0,72	0,73	0,72	0,71
9	0,94	0,92	0,93	0,90	0,90	0,89
10	0,63	0,60	0,60	0,62	0,62	0,58
11	0,53	0,53	0,61	0,58	0,54	0,54
12	0,95	0,92	1,01	1,00	0,97	0,95
13	0,87	0,93	0,98	0,99	0,91	0,90
14	0,79	0,78	0,84	0,84	0,81	0,79
15	0,68	0,62	0,71	0,72	0,65	0,65
16	0,86	0,78	0,87	0,84	0,79	0,82
17	0,98	1,00	1,05	1,09	0,97	0,92
18	0,94	0,98	0,92	0,96	1,00	1,00
19	0,78	0,77	0,75	0,71	0,76	0,74
20	0,91	0,91	0,85	0,82	0,85	0,87
21	1,03	1,00	0,96	0,94	0,94	0,96
22	1,07	1,04	0,98	0,95	1,02	0,97
23	1,02	0,99	1,00	0,92	0,98	0,95
24	1,02	0,95	0,91	0,89	0,93	0,92
25	0,96	0,94	0,91	0,88	0,97	0,92
26	0,79	0,77	0,73	0,74	0,76	0,74
27	0,74	0,69	0,64	0,63	0,69	0,65
28	0,59	0,57	0,59	0,59	0,54	0,55
29	0,80	0,73	0,80	0,77	0,78	0,74
30	0,93	0,83	0,94	0,86	0,85	0,82
31	0,87	0,78	0,80	0,77	0,77	0,74
32	0,61	0,54	0,55	0,55	0,54	0,55
33	1,17	1,11	1,12	1,06	1,08	1,07
34	0,84	0,76	0,75	0,73	0,72	0,73
35	0,58	0,56	0,60	0,61	0,58	0,56
36	0,73	0,70	0,77	0,75	0,70	0,71
37	0,54	0,57	0,58	0,62	0,54	0,55

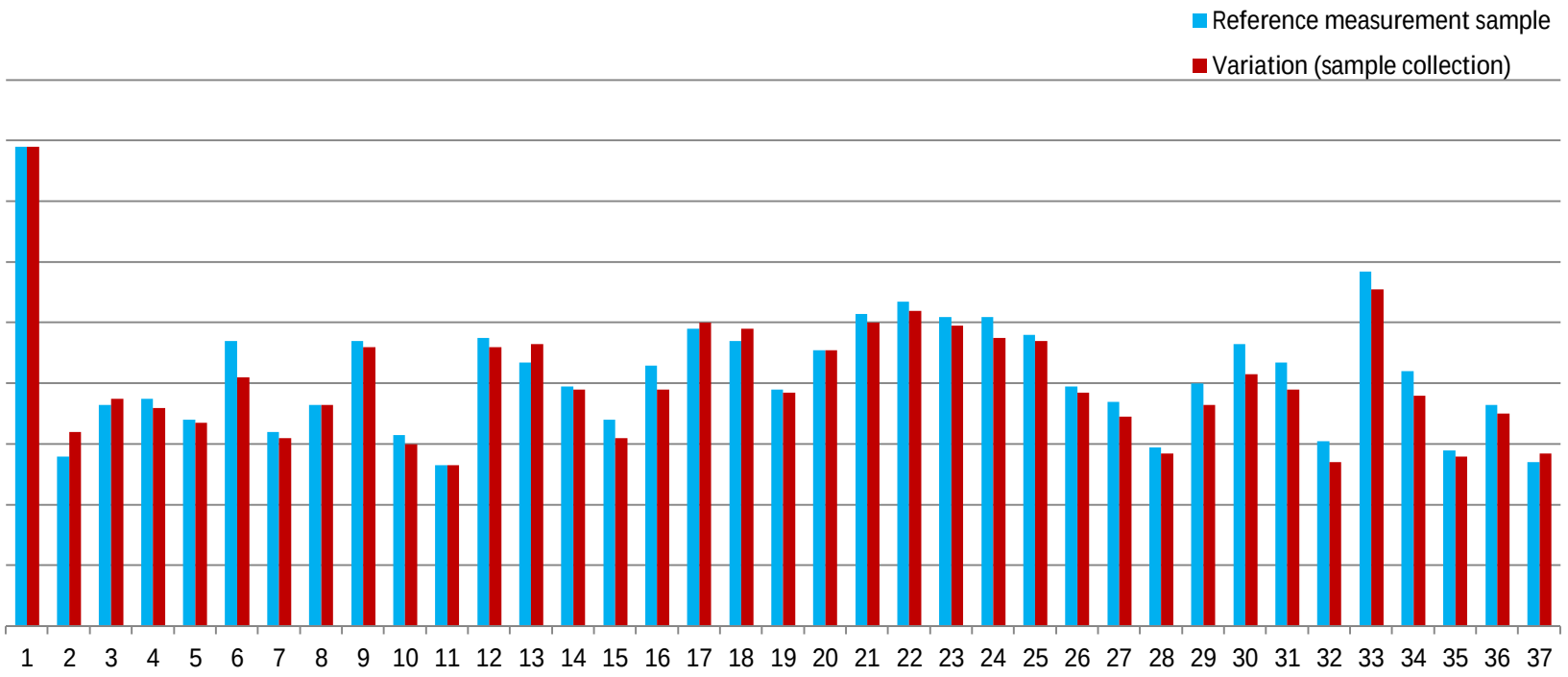


Figure 10 - Graphical illustration of the variation due to sample collection and handling, for Creatinine measurement.

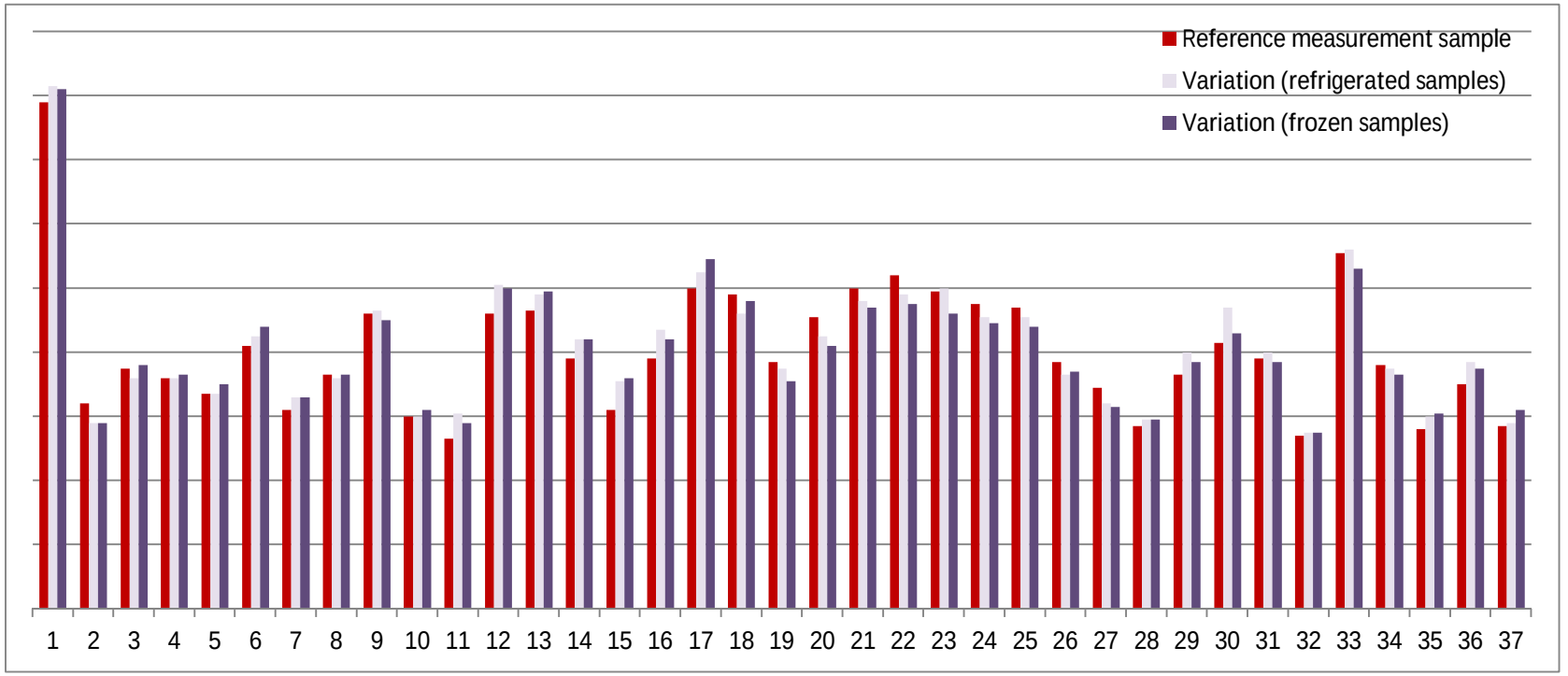


Figure 11 - Graphical illustration of the variation due to sample storage, for Creatinine measurement.

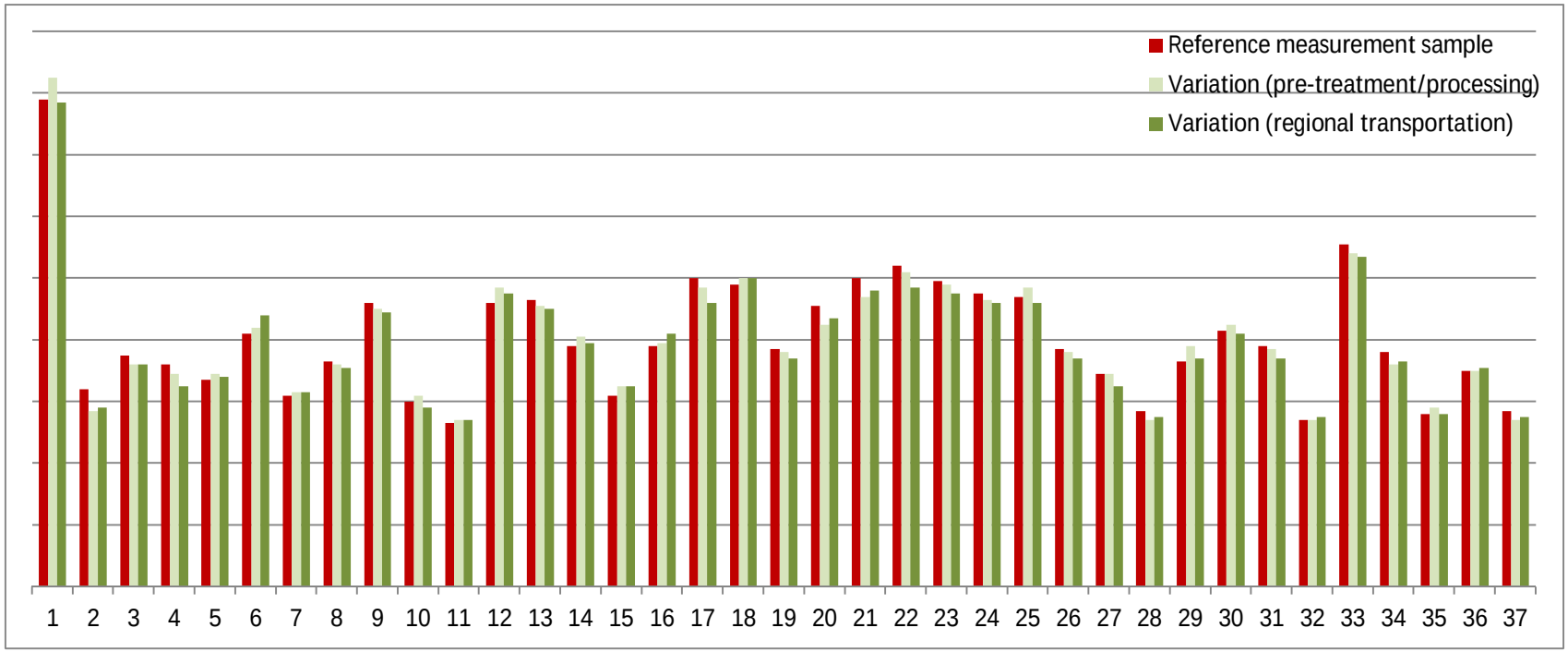


Figure 12 - Graphical illustration of the variation due to processing delay, for Creatinine measurement.

Table 6-11 – Glucose: Hexokinase/G-6-PDH Method

(Sample analyte data / mg/dL)

Samples	*A*	*B*	*B1*	*B2*	*C*	*D*
1	179	178	179	179	180	170
2	81	79	80	79	76	70
3	100	95	95	95	92	87
4	93	98	97	100	94	87
5	91	97	96	97	90	79
6	112	112	111	110	104	99
7	92	91	92	91	84	83
8	99	99	98	97	92	90
9	100	100	101	97	94	91
10	93	88	86	85	84	80
11	84	82	81	80	77	71
12	91	91	91	90	86	83
13	88	92	91	91	83	80
14	92	90	90	90	85	84
15	87	90	89	90	82	74
16	93	95	94	95	89	82
17	179	184	185	185	180	174
18	93	97	98	98	89	88
19	92	96	95	95	89	89
20	87	89	90	91	83	86
21	85	84	87	86	77	76
22	91	94	95	95	85	86
23	81	84	85	84	75	74
24	85	100	100	99	91	87
25	91	85	86	86	82	79
26	88	79	79	80	73	68
27	97	91	91	90	84	82
28	130	126	127	126	123	117
29	90	90	90	90	87	84
30	84	84	83	84	77	72
31	94	86	85	84	80	80
32	87	90	92	89	85	85
33	88	88	88	87	82	81
34	90	95	94	94	85	84
35	94	94	95	94	86	83
36	94	92	92	93	82	84
37	85	88	85	86	80	79

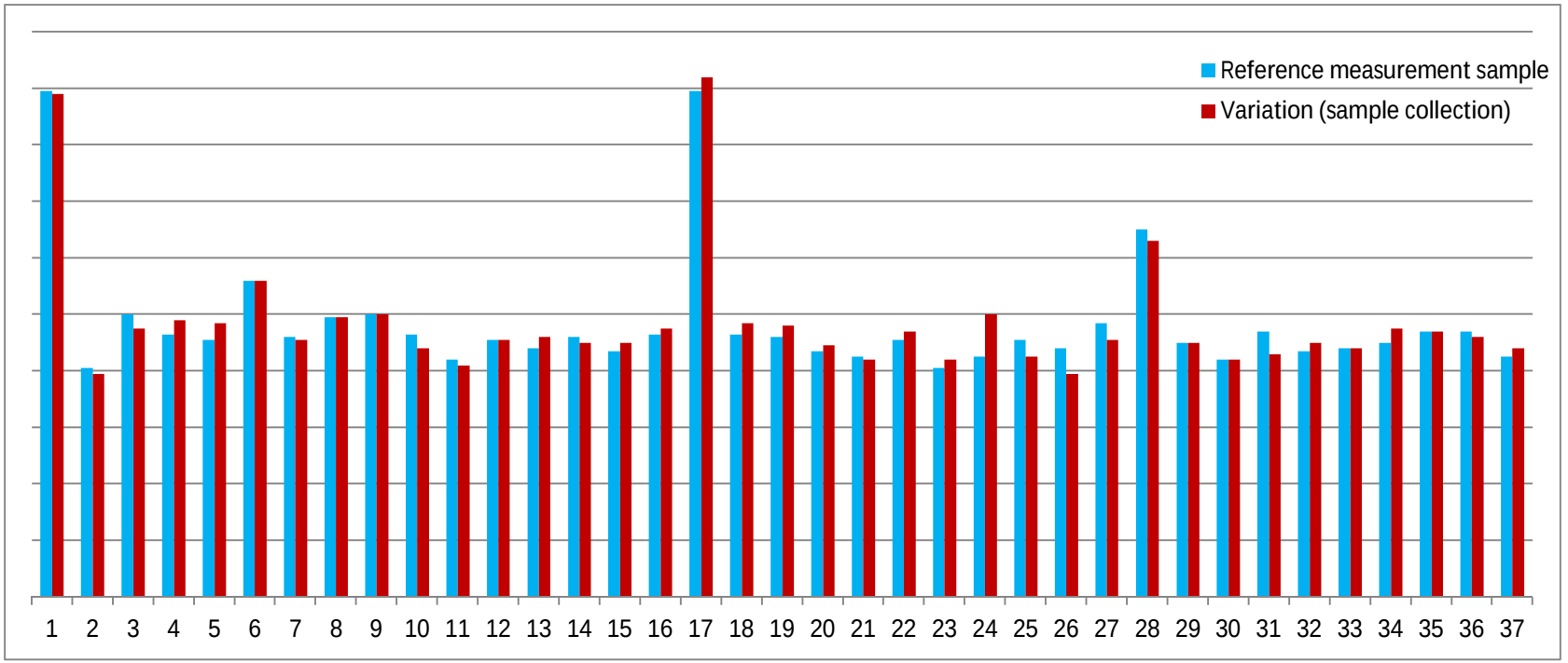


Figure 13 - Graphical illustration of the variation due to sample collection and handling, for Glucose measurement.

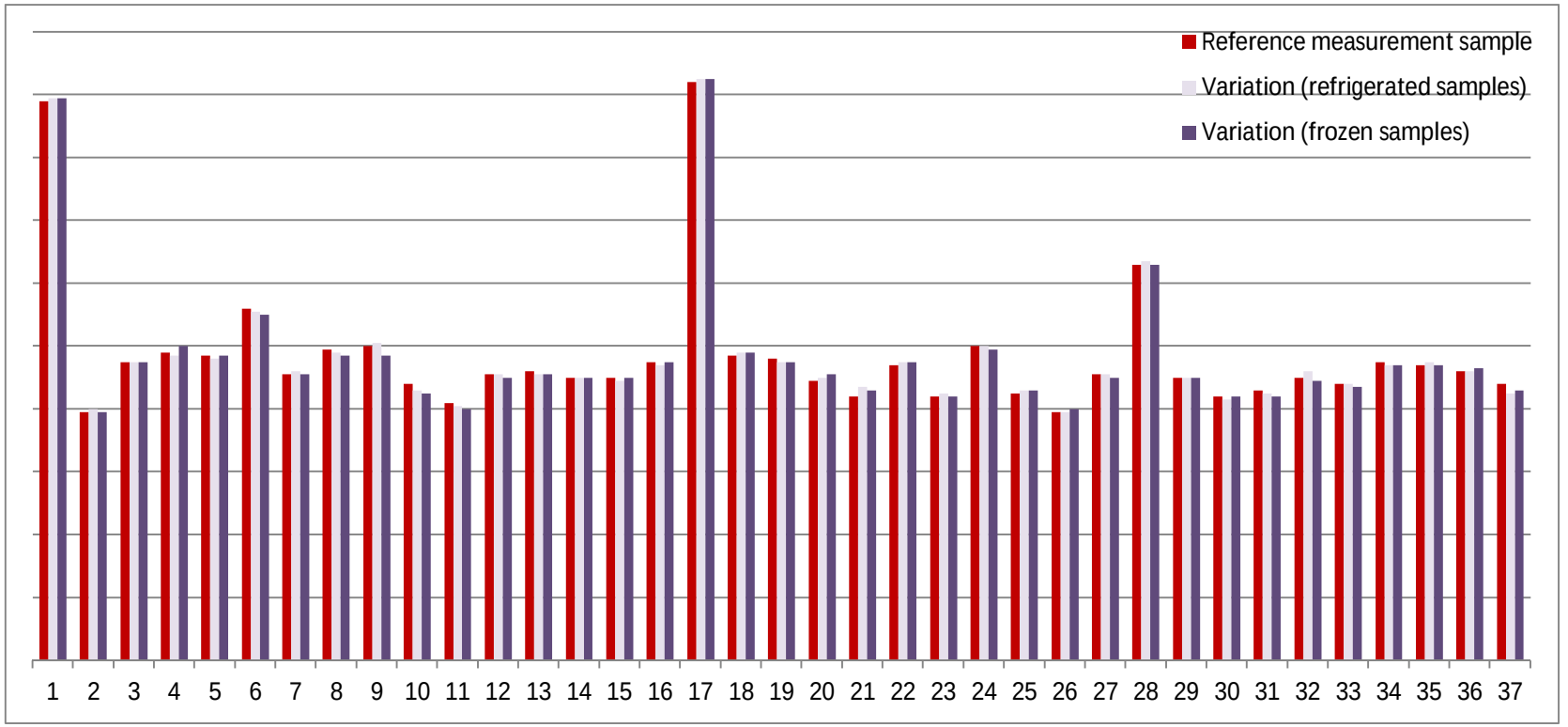


Figure 14 - Graphical illustration of the variation due to sample storage, for Glucose measurement.

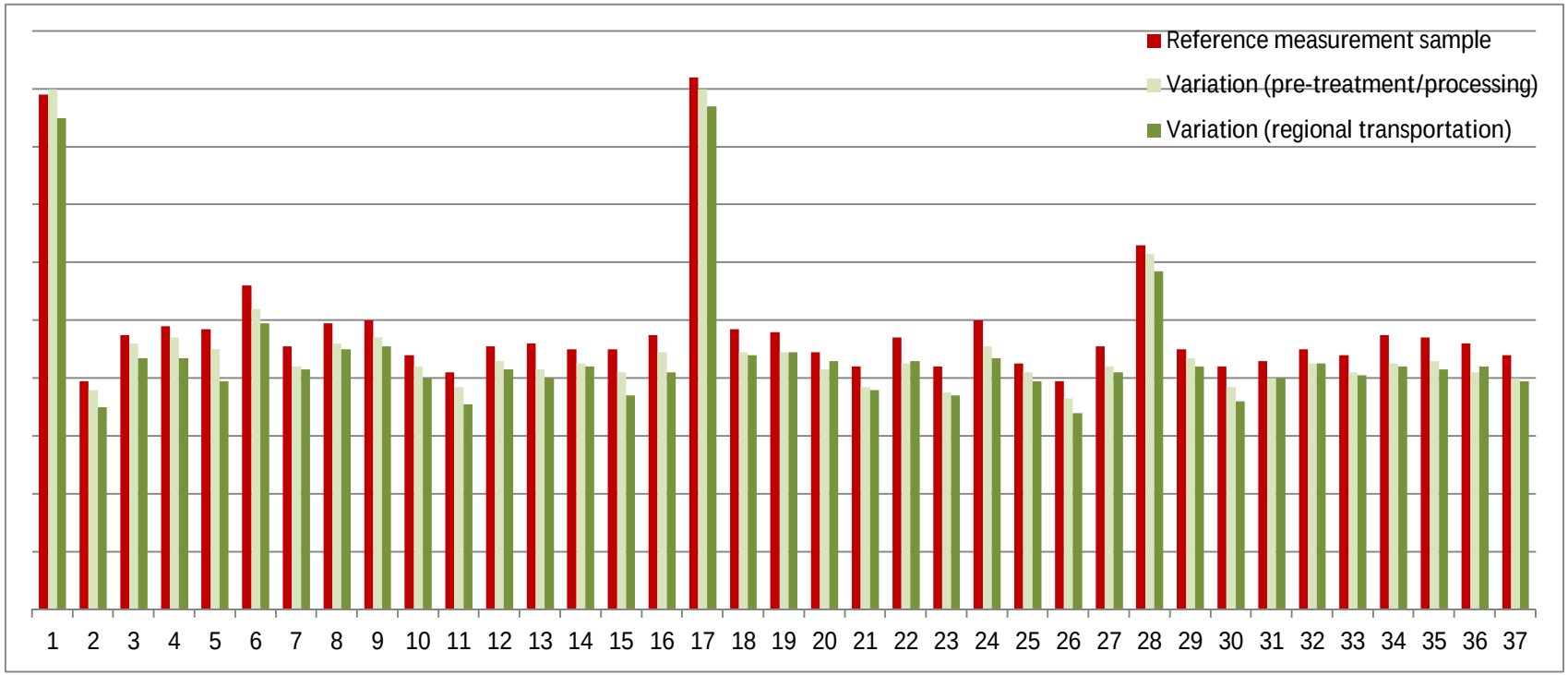


Figure 15 - Graphical illustration of the variation due to processing delay, for Glucose measurement.

Table 6-12 - Total Cholesterol measurement: CHOD/PAP Gen. 2 Method

(Sample analyte data / mg/dL)

Samples	*A*	*B*	*B1*	*B2*	*C*	*D*
1	180	174	180	181	176	178
2	239	234	238	237	233	239
3	190	193	196	199	194	194
4	270	258	268	265	268	266
5	204	198	204	199	200	200
6	218	216	216	215	216	219
7	186	185	185	182	187	186
8	206	204	202	200	205	204
9	237	244	238	210	243	243
10	177	175	171	160	171	173
11	185	190	186	177	186	187
12	278	276	274	278	280	279
13	176	177	176	174	177	180
14	229	227	223	226	228	230
15	168	159	160	158	160	159
16	153	151	152	151	153	153
17	184	189	183	183	187	187
18	200	195	198	196	196	197
19	156	154	157	159	155	156
20	231	228	231	229	228	229
21	254	251	253	248	249	249
22	270	275	275	272	271	276
23	131	126	127	127	127	129
24	194	185	189	186	185	187
25	212	210	210	214	210	213
26	162	160	161	158	160	163
27	194	174	172	174	172	172
28	125	122	120	120	122	122
29	199	195	196	188	196	198
30	268	262	260	261	260	265
31	178	175	176	173	171	173
32	199	198	199	196	196	200
33	182	178	182	179	178	177
34	216	206	207	203	205	206
35	185	185	186	185	187	186
36	204	208	210	209	204	207
37	186	186	188	189	186	187

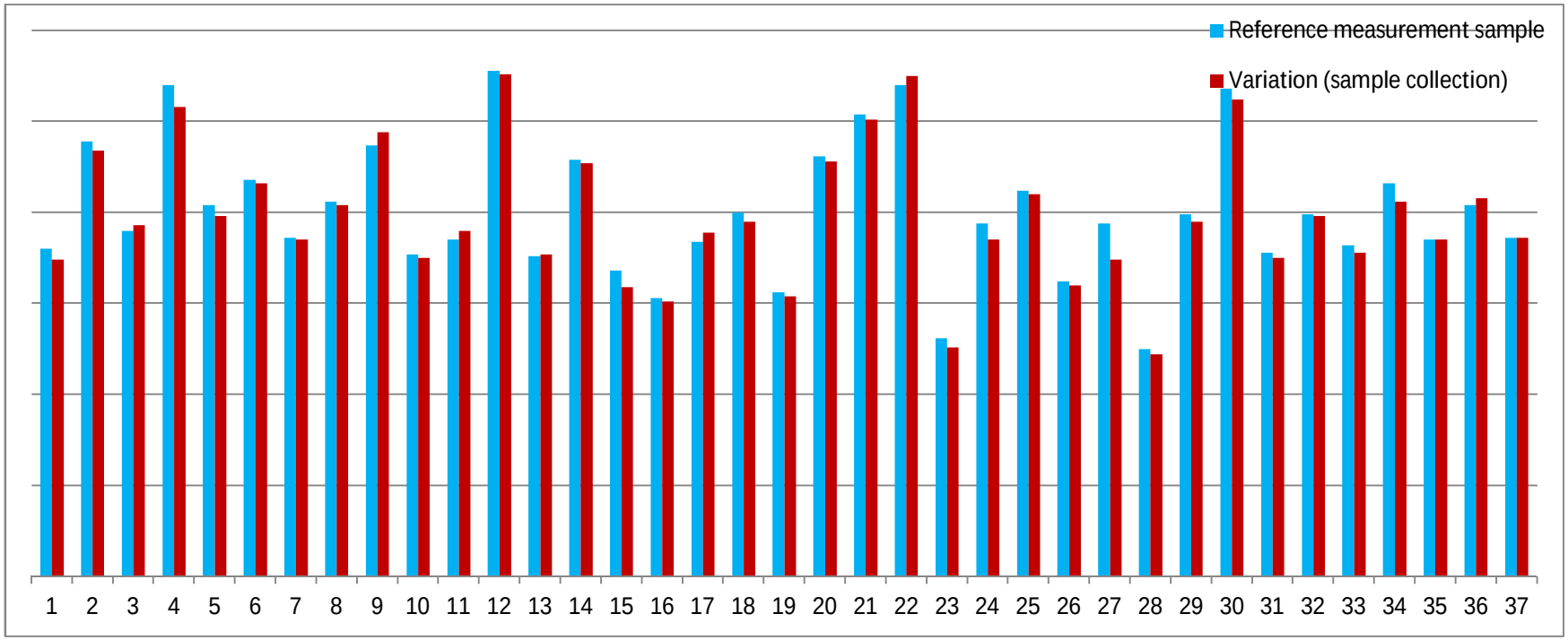


Figure 16 - Graphical illustration of the variation due to sample collection and handling, for T. Cholesterol measurement.

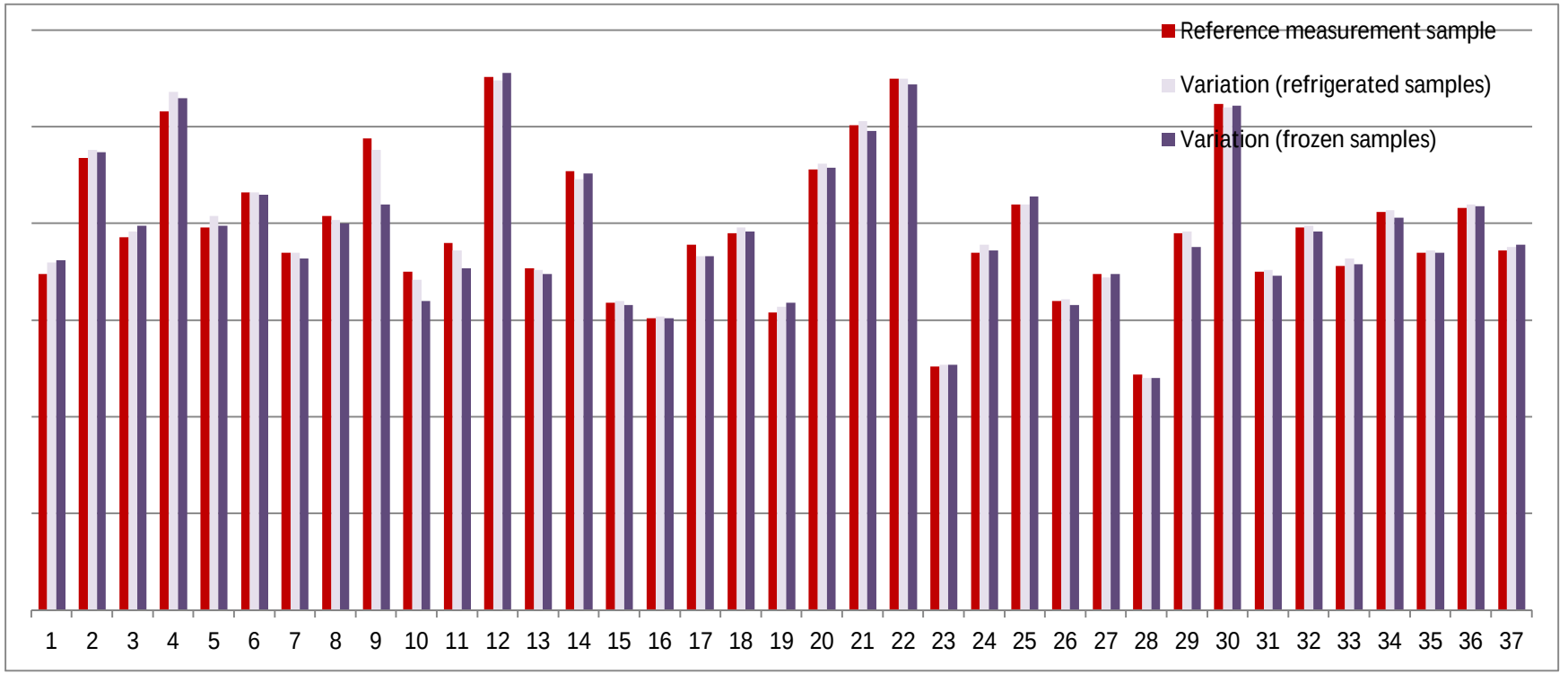


Figure 17 - Graphical illustration of the variation due to sample storage, for T. Cholesterol measurement.

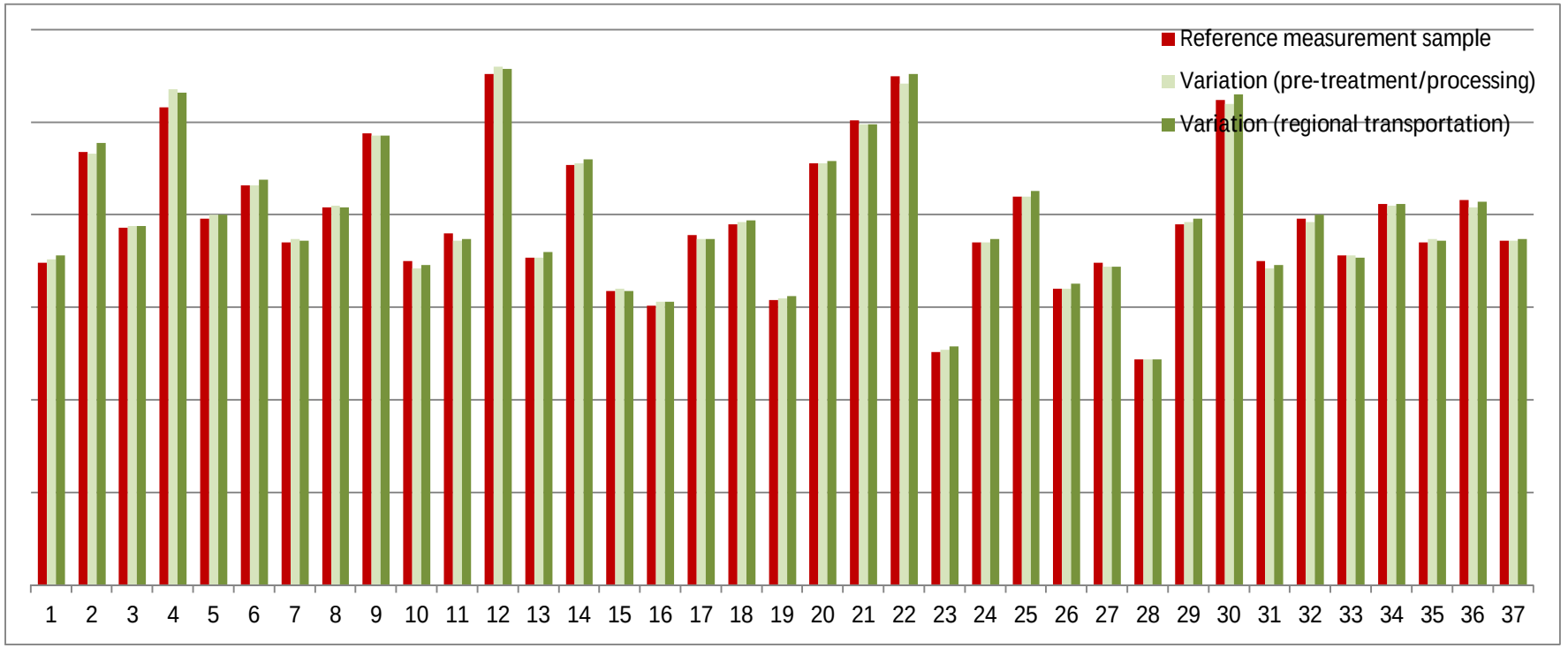


Figure 18 - Graphical illustration of the variation due to processing delay, for T. Cholesterol measurement.

6.3.2 Determination of Gnóstica's Pre-analytical Uncertainty

Applied the practical protocol to the 37 individuals that entered the study, the different uncertainty sources, within the pre-analytical variation, were assessed being were considered the paired results, between the reference samples and the different alternative/experimental procedures, to evaluate each variable in study.

The general coefficients of variation for each uncertainty source, were calculated (CV %; for B, B1, B2, C and D), corresponding to the total variation for each one, which included the analytical variation plus pre-analytical variation.

In Table 6-13 are presented the mean concentration values, for the 37 samples, for each category (B, B1, B2, C and D), of each analyte. In the same table the results for the general coefficients of variation are also shown, once again for each category, or pre-analytical uncertainty source.

Later, the coefficients of variation for the pre-analytical variation (CV_{Pre} %) were calculated for each quantity by subtracting the analytical coefficient of variation (CV_A), obtained from the day-to-day imprecision data from the laboratory's QMS, from the general CV calculated for each variable (B, B1, B2, C and D). resulting in each variability source's standard uncertainty (u).

These results are presented in Table 6-14, along with the values for the uncertainty estimate. The combined uncertainties ($u_{c\ pre}$) were obtained by the use of the general equation (2-1), and the final Expanded Pre-analytical Uncertainty (Upre) was calculated by applying equation (5-3), on page 55, with k=2, for a coverage probability of 95%.

Table 6-13 - Sample Analyte Data *(Mean Concentrations and CV's %)*

	Mean Concentration (mg/dL)						CV %				
Test / Analyte	A) Reference Measurement	B) Blood Collection	B1) Refrigerated Samples	B2) Frozen Samples	C) Sample Processing	D) Regional Transport	*B) Blood Collection*	*B1) Refrigerated Samples*	*B2) Frozen Samples*	*C) Sample Processing*	*D) Regional Transport*
Creatinine	0,83	*0,81*	*0,82*	*0,81*	*0,80*	*0,79*	***4,31***	***4,37***	***4,65***	***2,76***	***3,13***
Glucose	96,76	*97,11*	*97,11*	*96,81*	*91,00*	*87,78*	***3,26***	***0,85***	***0,97***	***4,94***	***7,48***
Total Cholesterol	200,70	*197,92*	*198,62*	*196,24*	*197,89*	*199,16*	***2,06***	***1,20***	***2,58***	***0,92***	***0,92***

Table 6-14 – Pre-analytical Uncertainty Components *(Combined and Expanded Pre-analytical Uncertainty)*

		Standard Uncertainty of					**Combined Preanalytical Uncertainty**	**Expanded Preanalytical Uncertainty**
Test / Analyte	**CV_A (%)**	*B) Blood Collection* **(uB %)**	*B1) Refrigerated Samples* **(uB1 %)**	*B2) Frozen Samples* **(uB2 %)**	*C) Sample Processing* **(uC %)**	*D) Regional Transportation* **(uD %)**	**$u_{c\ pre}$ (%)**	**U_{pre} (%)**
Creatinine	3,16	**1,15**	**1,21 (*)**	**1,49 (*)**	< CV_A	< CV_A	***1,15***	***2,30***
Glucose	1,50	**1,76**	< CV_A	< CV_A	**3,44**	**5,98 (*)**	***3,87***	***7,74***
Total Cholesterol	1,77	**0,29**	< CV_A	**0,81 (*)**	< CV_A	< CV_A	***0,29***	***0,58***

(*) - Not included in the calculations of Combined Uncertainties; **< CV_A** – Variation was smaller than the analytical coefficient of variation (CVA)

6.4 Measurement Uncertainty in the Medical Laboratory (II)

Having determined Gnóstica's pre-analytical uncertainty, the Measurement Uncertainty for this specific analytes could be updated. Moreover, having new data from the laboratory's QMS, this internal quality outcomes could also be used to obtain an update the whole estimate.

6.4.1 Application of the different MU estimate formulae - Gnóstica´s Pre-analytical Uncertainty (*2013 Update*)

The results in this section consist in the outcomes of combining the previous sections 5.2 and 5.3 methodology and results, respectively, updating the remaining uncertainty components with data from the current year QMS reports (referring to year 2013), for obtaining the new MU estimate, considering now the laboratory's own pre-analytical variability

Table 6-15 gathers the necessary data from of all the variability sources for the application of the different formulae to the analytes in study, namely the data on precision and trueness, once again including the two auto-analysers (*C1* and *C2*), and the calibrator's standard uncertainty values, considered as described in section 6.2.3, plus the pre-analytical from the internal study on section 6.3.

The updated MU estimate is presented on Table 6-16, where are also shown results for Total Error, as it was before, and displayed the outcomes from the application of both the MU formulae do the current data. As previously determined, MU-A formula uses the laboratory's IQC data and EQAS results, for imprecision and bias, and for the MU-B formula, are additionally considered the uncertainties associated to the calibrator and the pre-analytical. Again, it is also presented information on the quality performance specifications and goals, for matters of enabling comparing the different equations' outcomes

Table 6-15 – Data collection (Imprecision, Bias, Calibrator Uncertainty and Pre-analytical Uncertainty) for the implementation of the different MU estimating Formulae in study *(Data regarding the year 2013)*

Test		***Imprecision (CV%)*** *-Normal-*	***Imprecision (CV%)*** *-Pathological-*	***Trueness (|BIAS| %)***	***Calibrator Standard Uncertainty*** (CV %)	***Pre-analytical Standard Uncertainty*** (CV %)
Creatinine	***C1***	***4,83***	***3,87***	***3,22***	***0,71***	***1,15***
	C2	***3,15***	***2,45***	***0,33***		
Glucose	***C1***	***1,47***	***1,39***	***0,44***	***0,42***	***3,87***
	C2	***1,60***	***1,61***	***1,25***		
Total Cholesterol	***C1***	***2,48***	***1,75***	***0,90***	***0,44***	***0,29***
	C2	***2,23***	***1,78***	***0,93***		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

Table 6-16 - Estimated values for Total Error, MU Model-A and MU Model-B Formulae *(Data regarding the year 2013)*

Test		*Total Error -Normal- (%)*	*Total Error -Pathological- (%)*	*MU (A) -Normal- (%)*	*MU (A) -Pathological- (%)-*	*MU (B) -Normal- (%)*	*MU (B) -Pathological- (%)*	*TEa (%) European Table*	*TEa (%) CLIA Table*
Creatinine	**C1**	***12,9***	***10,9***	***11,6***	***10,1***	***10,0***	***8,2***	**8,9**	**15**
	C2	***6,6***	***5,2***	***6,3***	***4,9***	***6,9***	***5,6***		
Glucose	**C1**	***3,4***	***3,2***	***3,1***	***2,9***	***8,3***	***8,3***	**6,9**	**10**
	C2	***4,5***	***4,5***	***4,1***	***4,1***	***8,4***	***8,4***		
Total Cholesterol	**C1**	***5,9***	***4,4***	***5,3***	***3,9***	***5,1***	***3,7***	**8,5**	**10**
	C2	***5,4***	***4,5***	***4,8***	***4,0***	***4,6***	***3,7***		

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

6.5 IPAC’s Accreditation Auditing

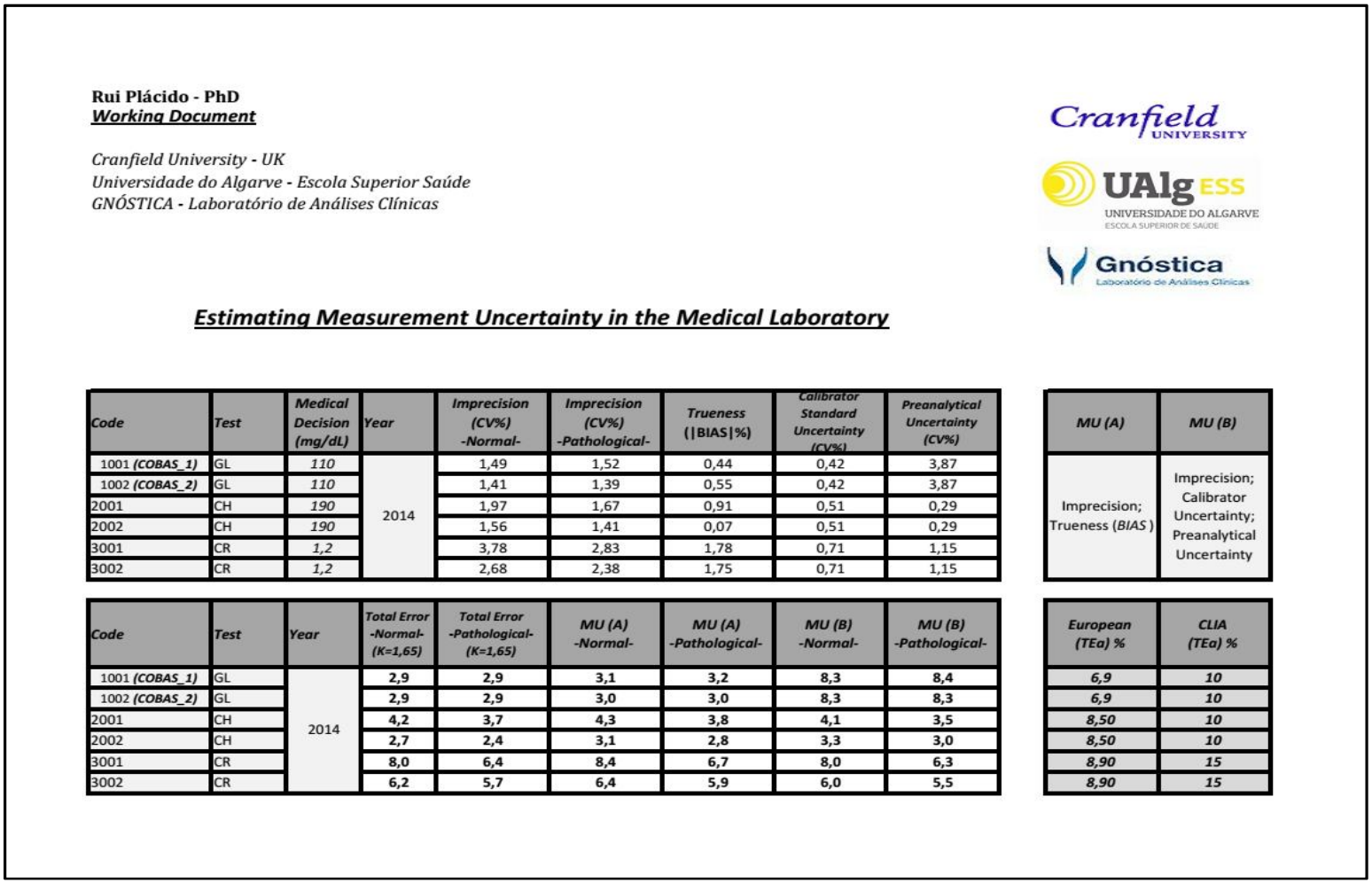
Following the update on the ISO 15189 Standard, in Portugal, the first auditing procedures under the new version (*NP EN ISO 15189:2014*) started in 2015.

As exposed, this new version of the ISO15189 does not suggest, it requires the measurement uncertainty estimate to be presented for the quantitative testing under the scope of the accreditation. At this time, just calculating the Total Error does not fulfil the standard’s, or the auditing team's, requirements anymore.

Having already realized the first tests with the formulas in study, with satisfactory results for the years of 2011 and 2013, to the concern of Gnóstica’s Quality Department, data from 2014 was collected and considered to be presented to the auditing team.

In this section, on Figure 19, are shown the first outcomes on MU, from the current study, presented for IPAC’s consideration regarding fulfilment of the accreditation requirements, which were then included on the report for the accreditation auditing.

For a matter of comparison, are included the results for Total Error, as well as for both of the MU formulae that are being applied in the study. With this, being the first auditing under the new requirements, which are still in the beginning of being implemented, it is also a moment of learning and discussion.



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Working Document

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GNÓSTICA - Laboratório de Análises Clínicas

Estimating Measurement Uncertainty in the Medical Laboratory

Code	Test	Medical Decision (mg/dL)	Year	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-	Trueness (|BIAS|%)	Calibrator Standard Uncertainty (CV%)	Preanalytical Uncertainty (CV%)
1001 *(COBAS_1)*	GL	*110*	2014	1,49	1,52	0,44	0,42	3,87
1002 *(COBAS_2)*	GL	*110*		1,41	1,39	0,55	0,42	3,87
2001	CH	*190*		1,97	1,67	0,91	0,51	0,29
2002	CH	*190*		1,56	1,41	0,07	0,51	0,29
3001	CR	*1,2*		3,78	2,83	1,78	0,71	1,15
3002	CR	*1,2*		2,68	2,38	1,75	0,71	1,15

MU (A)	MU (B)
Imprecision; Trueness (*BIAS*)	Imprecision; Calibrator Uncertainty; Preanalytical Uncertainty

Code	Test	Year	Total Error -Normal- (K=1,65)	Total Error -Pathological- (K=1,65)	MU (A) -Normal-	MU (A) -Pathological-	MU (B) -Normal-	MU (B) -Pathological-
1001 *(COBAS_1)*	GL	2014	2,9	2,9	3,1	3,2	8,3	8,4
1002 *(COBAS_2)*	GL		2,9	2,9	3,0	3,0	8,3	8,3
2001	CH		4,2	3,7	4,3	3,8	4,1	3,5
2002	CH		2,7	2,4	3,1	2,8	3,3	3,0
3001	CR		8,0	6,4	8,4	6,7	8,0	6,3
3002	CR		6,2	5,7	6,4	5,9	6,0	5,5

European (TEa) %	CLIA (TEa) %
6,9	*10*
6,9	*10*
8,50	*10*
8,50	*10*
8,90	*15*
8,90	*15*

Figure 19 Measurement Uncertainty report for IPAC's Accreditation Auditing

6.6 Measurement Uncertainty in the Medical Laboratory (III)

In this section are presented the results of the application of the final MU formula. After the studies with the laboratory's data regarding the years 2011 and 2013, in this final phase of the project, with updated data from 2015, the last and improved version of the MU formula was applied.

Being the primary objective, since the beginning of the project, to reach to a formula responding to Gnóstica's necessities regarding the accreditation requirements and procedures, imposed by the ISO 15189 standard and applied according to IPAC's auditing rules and criteria, the whole work was developed with that focus.

The final review and decisions were than operated in order to fulfil those intentions, considering not only the outcomes from sections 6.25.2 and 6.4, but most importantly the experience and feedback obtained from overcoming that first external assessment, after the procedures on section 6.5. This step extremely significant, since it revealed the positive outcomes of the work developed in this project, confirming the applicability and potential of successful implementation of the present proposed approach, and so fulfilling its goals.

This third application of the equation, still considering a last adjustment, or update, .was focused only on the chosen formula, being applied to the year's 2015 data, once again collecting information form the QMS, from the pre-analytical study previously developed and from the manufacturer's specifications.

6.6.1 Gnóstica's MU Estimate - Final Formula (MU-B)

In view of the above, decisions were made, considering MU-B model formula, represented in equation (5-2), on page 54, to be the one best fitting to the laboratory's objectives and to the study's purposes.

After demonstrating and quantifying the potential influence of the pre-analytical phase in the laboratory's measurements and results, on the studied analytes, was decided that this variation source should be considered and valued, being included in the Laboratory's MU estimate.

Assuming that the trueness control provided by the manufacturers, in the Traceability and Uncertainty Certificate (Roche) (Appendix J), represents the most appropriate approach for the quantitative expression of that performance characteristic, it was the best understanding that, being the required and necessary information regarding the traceability to higher order of the calibrators and the data on the uncertainty of that calibrating material available from the manufacturers, the MU estimate should also include those uncertainty values associated to the calibrators' assigned values.

Considering the above and since the mentioned manufacturer's specifications certificate for the calibrators used in the study's measurements, adequately state the traceability of the materials to the Reference Measurement Procedure (Isotope Dilution Mass Spectrometry - IDMS), indicating each calibrator's value and the correspondent associated uncertainty, was possible to meet the necessary conditions to implement applicate MU-B model equation (5-2).

Finally, bearing in mind recent publications' proposals in regards to the treatment of the random component [99, 101], enabling a simpler and straightforward application of the MU formula and its implementing in the laboratory's routine, was decided to calculate an overall value for the laboratory's intermediate precision component by the use of an global and inclusive CV (CV_{pooled}). This was achieved by combining the long term imprecisions from IQC (one year period), which were performed at normal and pathological concentration levels, by the use of equation (5-6).

Decided the final formula it was applied to the laboratory's with updated data, for a final assessment. This data, collected from the year 2015 exercise, is presented in Table 6-17. The relative standard uncertainties to include in the estimate, the results obtained for the measurement Combined Uncertainty and the final Expanded MU, for each studied analyte, are presented in Table 6-18.

Table 6-17 - Imprecision; Calibrator Uncertainty; Pre-analytical Uncertainty *(2015)*

Test		***Imprecision (CV %)*** *-Normal-*	***Imprecision (CV %)*** *-Pathological-*	***Calibrator Expanded Uncertainty*** (mg/dL)	***Calibrator Value*** (mg/dL)	***Pre-analytical Expanded Uncertainty*** (CV %)
Creatinine	**C1**	*5,13*	*4,02*	*0,0576*	*4,06*	*2,30*
	C2	*2,89*	*2,20*			
Glucose	**C1**	*1,42*	*1,54*	*1,63*	*193,0*	*7,74*
	C2	*1,55*	*1,43*			
Total Cholesterol	**C1**	*1,93*	*1,88*	*1,70*	*168,0*	*0,58*
	C2	*1,78*	*1,56*			

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

Table 6-18 – Data; Combined and Expanded Uncertainty (2015)

Test		***Imprecision Pooled*** (CV %)	***Calibrator Standard Uncertainty*** (CV %)	***Pre-analytical Standard Uncertainty*** (CV %)	***MU (B) - Combined Uncertainty -***	***MU (B) - Expanded Uncertainty -***
Creatinine	**C1**	**4,60**	***0,71***	***1,15***	**4,80**	**9,60**
	C2	**2,57**			**2,90**	**5,80**
Glucose	**C1**	**1,48**	***0,42***	***3,87***	**4,16**	**8,32**
	C2	**1,49**			**4,17**	**8,34**
Total Cholesterol	**C1**	**1,91**	***0,51***	***0,29***	**2,00**	**4,00**
	C2	**1,67**			**1,77**	**3,54**

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

6.6.2 Standard Uncertainties' Index

In this last phase, to evince and perceive the effective influence of each uncertainty source, which are being considered in the MU estimate, their relative weights were calculated, regarding the final result. So, the "index" indicates the relative contributions to the combined final result. The values obtained are presented in Table 6-19, showing each considered variation source's relative standard uncertainty and the respective index, regarding the combined uncertainties, for each of the components. These outcomes are also graphically displayed in Figure 20 below, to promote a more visual perception of the findings.

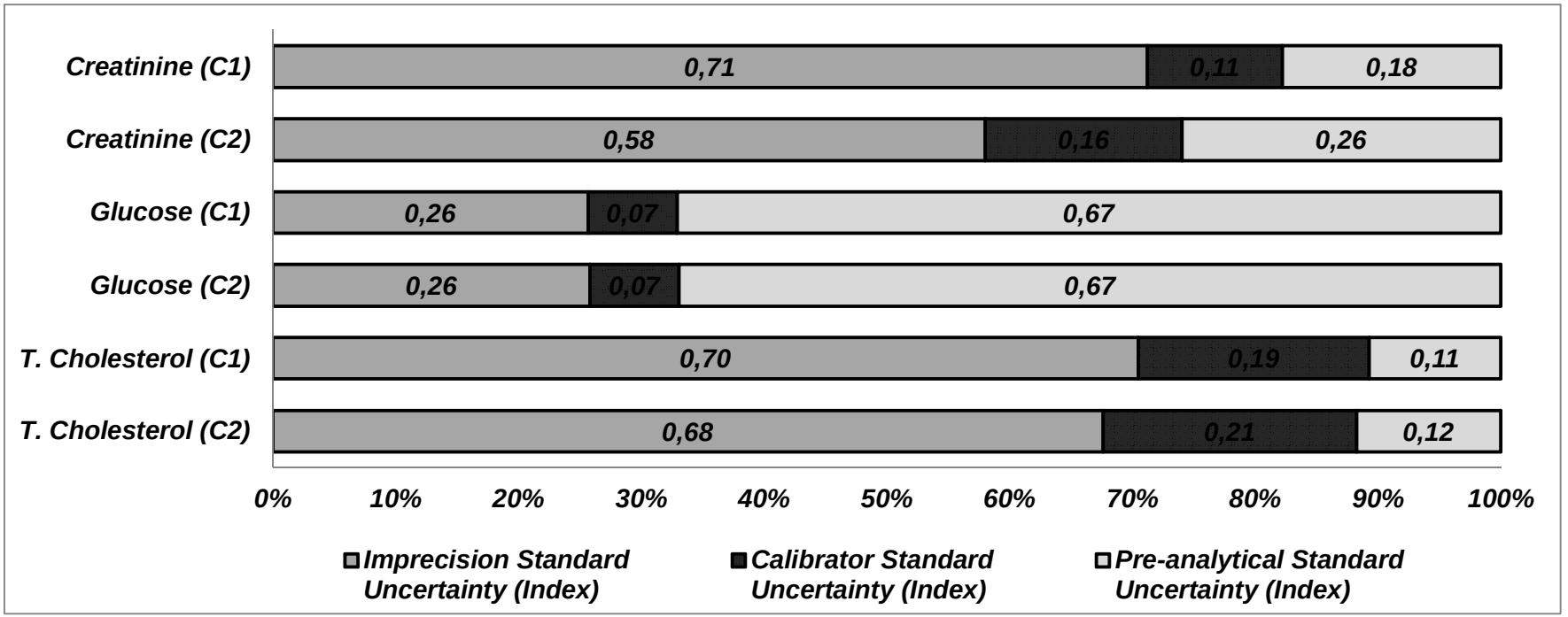
Determining this Uncertainty Index allows identifying the components that most contribute to the MU value, which consequently have the most potential of causing any variation on the final measurements results. This evaluation provides important information, revealing the measurements' probable weak points, in terms of variability of its results, accomplishing so one of the possible benefits of implementing a protocol for estimating measurement uncertainties.

The added value of these outcomes lies on enabling the laboratory with a tool that potentiates its power and ability to increase the reliability of their results. The laboratory, by analysing these findings, valuing its evidences and acting in consequence, may implement the necessary measures to enhance its procedures and methods, being able to improve the quality of the measurements performed and of the services offered to its users.

Table 6-19 Standard Uncertainties' Index

Test		*Imprecision Standard Uncertainty* (CV %)	*Calibrator Standard Uncertainty* (CV %)	*Pre-analytical Standard Uncertainty* (CV %)	*Imprecision Standard Uncertainty* (Index)	*Calibrator Standard Uncertainty* (Index)	*Pre-analytical Standard Uncertainty* (Index)
Creatinine	***C1***	***4,60***	***0,71***	***1,15***	***0,71***	***0,11***	***0,18***
	C2	***2,57***			***0,58***	***0,16***	***0,26***
Glucose	***C1***	***1,48***	***0,42***	***3,87***	***0,26***	***0,07***	***0,67***
	C2	***1,49***			***0,26***	***0,07***	***0,67***
T. Cholesterol	***C1***	***1,91***	***0,51***	***0,29***	***0,70***	***0,19***	***0,11***
	C2	***1,67***			***0,68***	***0,21***	***0,12***

C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2



C1 - Cobas®6000 - c501 Module: Equipment 1; ***C2*** - Cobas®6000 - c501 Module: Equipment 2

Figure 20 Graphical illustration of Standard Uncertainties Index

6.7 MU Estimate Final Tool:
Gnóstica's Working Spreadsheets (Templates)

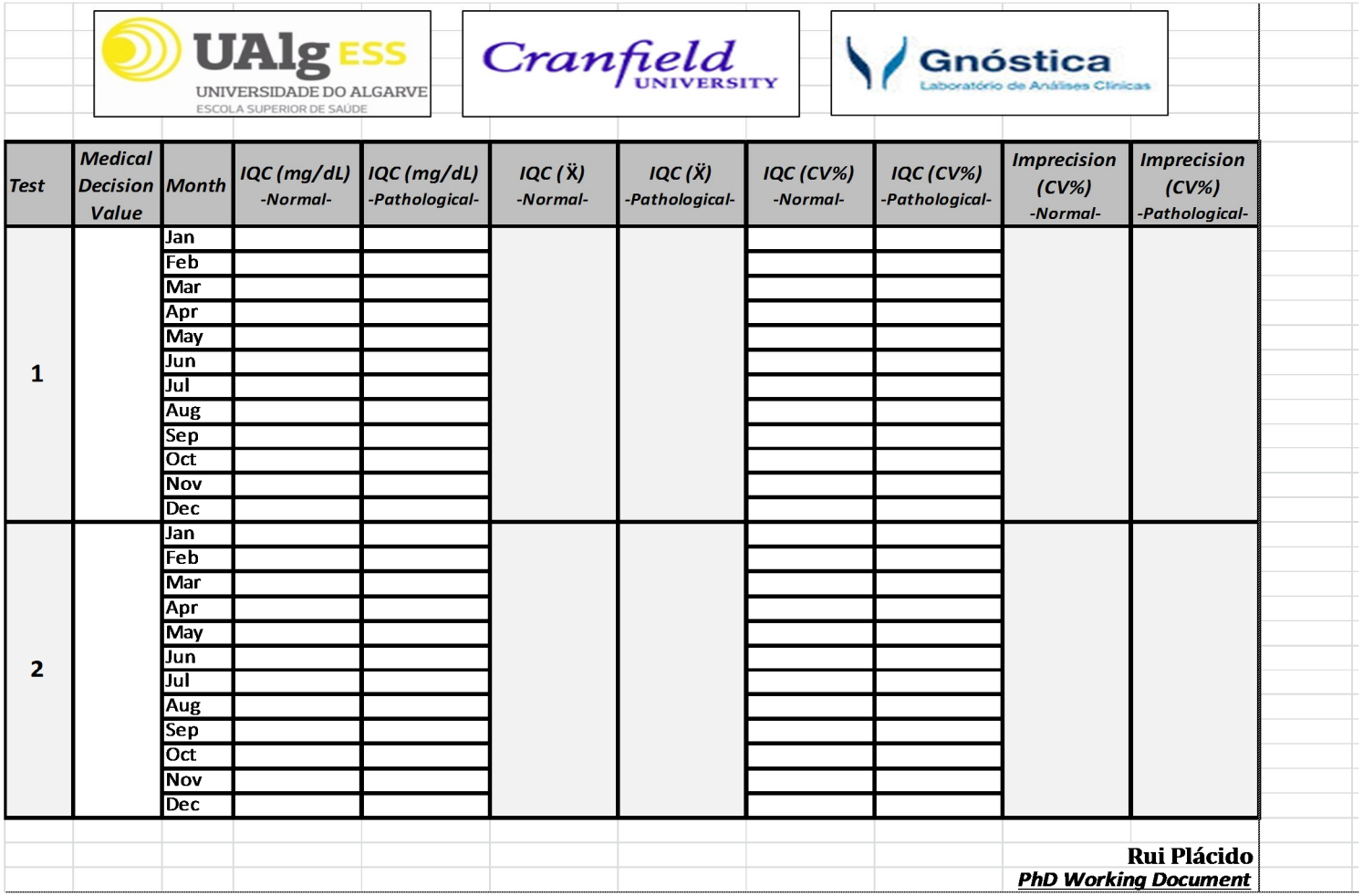
Towards the objective of an effective, but simple, way of proceeding with the implementation of MU estimate in the laboratory's routine, was created a practical tool to be introduced amongst the other quality management procedures.

That was accomplished by developing an Excel® (*Microsoft*) spreadsheet, contemplating the uncertainty sources considered, which allowed to use and update the monthly or annual values already being collected within laboratory's QMS, along with other data necessary, concerning the formula application, enabling to easily obtain the estimates of the Laboratory's Measurement Uncertainty for the results of the selected tests or measurements.

Concerning the data introduction, the tables are easy to replicate, allowing adding different tests to it. It was also predicted the possibility of considering the introduction of other uncertainty sources, being automatically included on the formula and combined in the final estimate.

The following section displays simple snapshots of the working spreadsheet, on Figure 21, Figure 22 and Figure 23, regarding respectively the introduction of data from the laboratory's intermediate precision (IQC); from the systematic variation source (EQAS), obtaining the measurements' bias (if the circumstances so require, as addressed on the previous sections); and the final sheet, which will deliver the MU Estimates, and contemplates an inserted data summary compilation, with a reserved area for the input of the last necessary data.

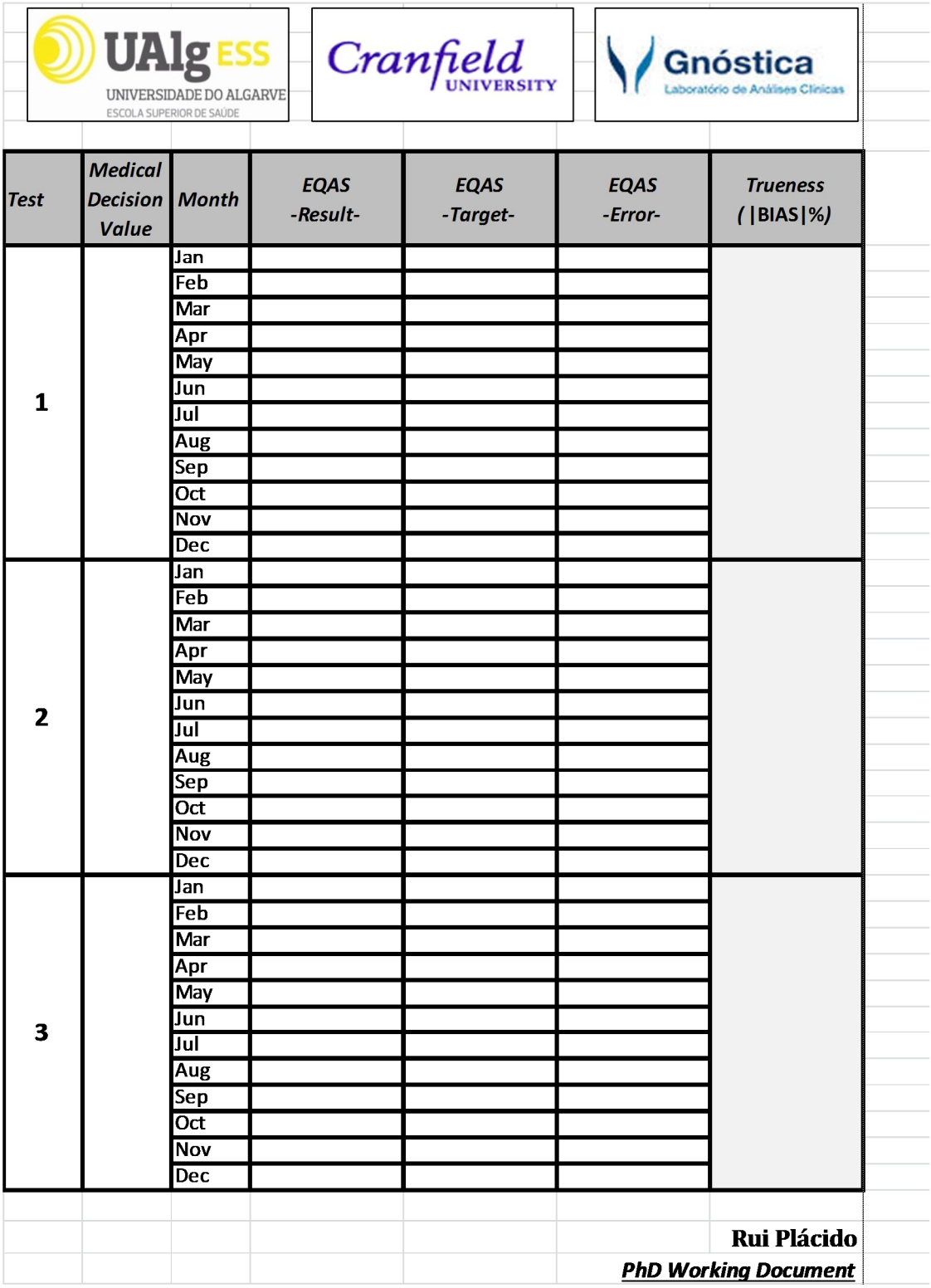
In every worksheet, the white columns represent data input areas and the darker columns (grey) indicate an outcome for each specific identified parameter. These dark areas are blocked to the user, such as the formulae area, which do not show, not being accessible in the working area environment.



Test	Medical Decision Value	Month	IQC (mg/dL) -Normal-	IQC (mg/dL) -Pathological-	IQC ($\bar{X}$) -Normal-	IQC ($\bar{X}$) -Pathological-	IQC (CV%) -Normal-	IQC (CV%) -Pathological-	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-
1		Jan								
		Feb								
		Mar								
		Apr								
		May								
		Jun								
		Jul								
		Aug								
		Sep								
		Oct								
		Nov								
		Dec								
2		Jan								
		Feb								
		Mar								
		Apr								
		May								
		Jun								
		Jul								
		Aug								
		Sep								
		Oct								
		Nov								
		Dec								

Rui Plácido
PhD Working Document

Figure 21 Random Variability Component (IQC data, corresponding to the laboratory's intermediate precision determination)



Test	Medical Decision Value	Month	EQAS -Result-	EQAS -Target-	EQAS -Error-	Trueness (|BIAS|%)
1		Jan				
		Feb				
		Mar				
		Apr				
		May				
		Jun				
		Jul				
		Aug				
		Sep				
		Oct				
		Nov				
		Dec				
2		Jan				
		Feb				
		Mar				
		Apr				
		May				
		Jun				
		Jul				
		Aug				
		Sep				
		Oct				
		Nov				
		Dec				
3		Jan				
		Feb				
		Mar				
		Apr				
		May				
		Jun				
		Jul				
		Aug				
		Sep				
		Oct				
		Nov				
		Dec				

Figure 22 Systematic Variability Component: Laboratory's Bias determination

UAlg ESS
UNIVERSIDADE DO ALGARVE
ESCOLA SUPERIOR DE SAÚDE

Cranfield UNIVERSITY

Gnóstica
Laboratório de Análises Clínicas

Measurement Uncertainty Estimation

Test	Medical Decision Value	Random Variability			Systematic Variability (*Trueness*)					Preanalytical Uncertainty (CV%)	Other Uncertainty Sources	Combined Uncertainty (u_c)	Expanded Uncertainty (U)
		Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-	Imprecision (Pooled CV%)	Calibrator Expanded Uncertainty (mg/dL)	Calibrator Value (mg/dL)	Calibrator Standard Uncertainty (CV%)	|BIAS|%	U_{BIAS}				
1													
2													
3													
4													
5													
...													
...													
...													

Measurement Uncertainty (MU)	Uncertainty Sources/Components:
	Random Analytical Sources ($U_{Imprecision}$); Systematic Analytical Sources (Trueness: U_{CAL}; U_{bias}); Pre-analitycal Uncertainty; Other Uncertainty Sources

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Figure 23 MU Estimate Worksheet

7 DISCUSSION

7.1 Quality Indicators

Quality indicators are important management tools that can be used to evaluate the organization's overall performance and the effectiveness of implemented policies. The observation in detail of specific aspects of performance allows to analyse and follow not only the evolution of the quality of the services offered by the laboratory but also the users perception of it, as well as its consequences in terms of working volume growth (clients and tests).

Usually, the assembled information, sometimes including suggestions directly from the users, reveals improvement opportunities, becoming starting points to progress and upgrade in the pursuit for a better service with higher quality standards.

Based on the Quality Management System data, was performed a retrospective study with the objective of evaluate the available quality indicators. During the period covered by this study could be observed the evolution of the Quality Management System while the laboratory was passing through two different processes: Certification by the standard: ISO 9001:2000 (in 2004), and later, Accreditation by the standard NP EN ISO15189:2007 (in 2009).

First of all it is important to emphasize that throughout these years, following the certification/accreditation processes, it is verified a considerable growth in the number of inscriptions in the laboratory, resulting in an increase from 36640 (2003) to 84327 users/patients (2010) and in a consequent increase in the number of tests/analysis from 330441 to 810432, during the same period.

During this same time the waiting time in the Laboratory was reduced to 9,7 minutes (2010), after having reached a maximum record in 2006, with over 25 minutes. Also the number of repetitions of sample collecting decrease from 1,2% (2003) to 0,7% (2010) and there was a great improvement in repeat testing, dropping from 6,1% (2003), to 1,0% (2010);

On the External Quality Assessment Programs, comparing the percentage of correct results in 2003 (20 different schemes) and in 2009 (29 programs completed), there was an important improvement from 90% to 96%;

In an overall analysis, it's important to highlight the record of 0,03% non-conformities (2010), in comparison with the values of the previous years of 0,12%; 0,17% or 0,18% (2003; 2004/2006; 2008). The majority of non-conformities (80% in 2010) have had origin in the reception, revealing a possible weak point and an opportunity to improve. These numbers resemble the findings of a recent study on pre-analytical errors, which reported booking-in errors as the most common errors reported by the participating laboratories, with a 70,1% rate [103].

But, in fact, if considered the increase in the working volume, it can be verified the quality system's benefices at this level. When looking to the absolute number of NC it can noticed the decrease of its values, shown not only in the total of non-conformities but also in the reception registries, where the progress exist, although there is also still room for improvement. It is also possible to infer about the improvement in the remaining procedures of the pre-analytical phase (excluding the patients registry at the reception), as well as in the analytical and post-analytical phases, where the 5 NC detected result in 0,006% of NC, considering the total of 84327 users/patients.

Similarly, relating with the increase in the working volume, are considered as positive the results of the user's overall satisfaction, as well as the fulfilment of the delivery dates and of the percentage of results delivered in the same day.

These indicators, which maintained the same level of results, apparently not showing neither improvement nor worsening, end up revealing the stability of the implemented procedures and of the quality system established, taking into account the growth in users/patients and tests/analysis requested and executed.

The QIs observed reveal real improvements brought by the Certification, later reinforced by the Accreditation process. There is still much work ahead and improvements to achieve, but this seems to be the way in the search for Total Quality, by monitoring and valuing the quality indicators assessed, also by listening to the users and taking upgrade initiatives and implementing improvement projects and actions, aiming to achieve the highest standards and recommendations for quality and quality systems management.

An example is the implementation of user-focused policies, responding to their criticisms and suggestions, which allowed the application of corrective actions, namely in the reduction of waiting time, transforming a weakness in a strong point. It also could be observed the increase in the number of users and analysis per year, to which can be added a reduction of costs due to the decrease in the number of test and sample collection repeating (non-quality costs).

Thus, the present study shows indications that the implementation of ISO Standards for Quality Systems in Clinical Laboratories (ISO9001 and ISO15189) is very important to monitor the laboratory's overall performance and contribution to patient care, providing tools to improve the quality of services, processes and results.

The objective was not to exhaustively analyse and evaluate the performance of each indicator, as it merely aimed to perceive and demonstrate the advantages and the evolution brought by the implementation of the quality system, as well as the improvements arising from the upgrade to Accreditation by the ISO 15189 standard, which are evident in view of the presented data.

Being also an essential requirement in accreditation processes, it is important that laboratories not only adopt strategies and tools to enable monitoring QIs, but also to identify key quality indicators, to harmonize its evaluation procedures and to establish goals and performance levels for its management.

This work as to be promoted and enhanced by international bodies and by the scientific associations, as it is already being done, “yet with no consensus on the use of QIs focussing on all steps of the laboratory total testing process (TTP), and further research in this area is required” [104].

The IFCC has created a working group on ‘‘Laboratory Errors and Patient Safety’’ (WG-LEPS) which, focused in reduce the errors in laboratory testing, developed a series of Quality Indicators, specifically designed for clinical laboratories, aiming to the development of a common reporting system for clinical laboratories based on standardized data collection, and to define state-of-the-art and Quality Specifications (QSs) for each QI [104].

On behalf of the IFCC Working Group on ‘‘Laboratory Errors and Patient Safety’’ (WG-LEPS), Sciacovelli, L., *et al.*, published a preliminary report entitled “*Quality Indicators in Laboratory Medicine: from theory to practice*”, suggesting that the implementation of QIs through EQAS programmes as tools to monitor and control the total testing process would lead to the development and improvement of specific QIs for clinical laboratories as well as quality specifications for each one of them. This would not only allow the identification of potential risks to patient safety, but also the sharing of practices that can help reduce/prevent errors, enabling the dissemination of improvement measures.

With special focus on the extra-analytical phases Hawkins, R. [105], elected the management of these particular procedures in the total testing process as the “next challenge for laboratory medicine”, valorising the publication of QIs and proposed QSs by the IFCC WG-LEPS, as it provides “useful benchmarking data for laboratories embarking on extra-analytical quality improvement programmes”.

These statements find sequence in the publications by Plebani, M., *et al.*, and Sciacovelli, L., *et al.*, on performance criteria and quality indicators regarding the pre-analytical phase and the post-analytical phase respectively [106, 107].

After stating the necessity of consensus in the implementation and evaluation of quality indicators in laboratory diagnostics, as well as the harmonization of its monitoring practices, as being essential to drive real improvements in laboratory medicine and health care [108], and while “there is no consensus for the production of joint recommendations focusing on the adoption of universal QIs and common terminology in the total testing process”, Plebani, M., *et al.*, have published an opinion paper [109], following the work of the IFCC WG-LEPS, highlighting the preliminary consensus achieved in the road to the harmonization of quality indicators in laboratory medicine. It is presented a revised list of QIs developed by IFCC WG-LEPS, and proposed definitions to all the QIs with examples to allow the clarification and help to the comprehension of the meaning of each QI.

In conclusion it is reinforced that “the measurement and monitoring of QIs in laboratory medicine serve many purposes: 1) document the quality of the service provided; 2) improve performance and patient safety; 3) make comparison (benchmarking) over time between laboratories; 4) make judgments and set priorities (corrective actions to be performed); and 5) support accountability, quality improvement and accreditation” [109].

7.2 Measurement Uncertainty in the Medical Laboratory

7.2.1 Application of the different MU estimate

As exposed, in Portugal, for the demonstration of determination of measurement uncertainty during the accreditation process for clinical laboratories it was accepted that the laboratories presented the calculations of Total Error. Once again, as stated, this study was developed in a Portuguese ISO15189 Accredited Medical Laboratory, having all the procedures for the assessed analytes being included in the mentioned process and are all accredited as well. In the accreditation process it is a mandatory requisite the proof and demonstration of participation in External Quality Assessment Schemes (EQAS), as it is the record of good performance in those schemes. All of these premises are, in this case, fulfilled.

The results from the Quality Management System were considered, particularly the data from the Internal Quality Control (IQC) and from the External Quality Assessment (EQAS). From here, it was also possible to calculate the Total Error (TE), which was used in Portuguese Laboratories, at this time (2011/2012), to assess and validate the laboratory methods, as well as to make proof of technical capability and competence in the process of Accreditation, as stated earlier. These results were compared against the reference values for allowable total error (TEa), from international tables and guidelines, such as the "Desirable Biological Variation Database Specifications" [17] or the "CLIA regulations table" [18]. For this purpose, were considered three different analytes, being Total Cholesterol, Creatinine and Glucose, all measured in human serum. There are two identical *Cobas*® 6000 – c501 module (Roche®), so where are presented the results obtained for both of them, as it was information of value to the laboratory and for its quality management department, allowing eventually further comparison material and possibilities. As mentioned, both work under the same conditions, handled by the same

technicians with the same reagents, controls and calibration material (Cobas® c501 / c502 / c311 / c701 / c702 - C.a.s.f.), from Roche®.

Combining these data allowed applying directly the proposed *Model-A* formula (5-1). Plus, by gathering information on the values of uncertainty assigned to the calibrator, from the manufacturers certificate (Roche®), and of the pre-metrological variability for these same analytes, from the literature [85], it was possible to apply Model-B (5-2), formula both presented earlier in Section 5.2.

By comparing the outcomes with quality goals and performance specifications from both of the Tables, the European and CLIA's, it is possible to affirm that for total cholesterol all the results are within both limits. In the Creatinine's case, all the values are within the less tight values of CLIA's Table, failing the limits of the European Table for TE and MU-A, while for MU-B the results for the pathological (high) level fall within these limits, while the ones for normal control level fail the desirable specification limits. As for Glucose, the values of TE and MU-A all meet the goals of the European and CLIA's Tables, while the results for MU-B also fails to comply with the limits required by the European Table.

However, this is a rather relative exercise, bearing in mind that the implementation of ISO 15189 standard, although requiring to the medical laboratories measurements uncertainty estimate, does not provide any guidance or specifications regarding acceptable limits under which the measurement uncertainty should be determined and afterwards evaluated.

For this purpose, in an opinion paper regarding "*Permissible limits for uncertainty of measurement in laboratory medicine*", Haeckel, *et al.*, considering that "the most common concept for establishing permissible uncertainty limits is to relate them on biological variation defining the rate of false positive results or to base the limits on the state-of-the-art", presented a proposal of an algorithm to deliver the necessary performance goals. Claiming that it "can be applied to all measurands and consider any quantity to be assured", the approach tries to reconcile the biological variation concept, the GUM uncertainty model and the technical state-of-the-art [110].

Recently, also Farrance, *et al.*, raised the question on “what performance specifications (quality goals) can supporters of the GUM approach use as a guide to actual or comparative performance?” Then, providing a possible answer, the authors reference Ricos’ “Desirable Biological Variation Database Specifications Table” [17], stating that, being biological variation and the total error independent entities, biological variation can be applied to compare and evaluate measurement uncertainty outcomes, just as it is used in the assessment of the total error determinations and principles [98].

In addition to the desirable specifications for Total Error, Ricos’ Table presents data for intra-individual (or within-subject - CV_W) and inter-individual (or between-subject - CV_b) biological variation, which are the basis for the remaining specifications presented. Yet without specific performance limits defined for measurement uncertainty, besides the allowable total error values, considering bias and precision as components, have been proposed to use analytical goals for imprecision, based on intra-individual biological variation to assess uncertainty outcomes [33, 111].

Nevertheless, assuming that comparing measurement uncertainty with biological variation derived limits, considering that the possible variation interval delivered by the uncertainty estimate for a given a test result should be comprised within permissible limits set on the light of that concept, which is well implemented and studied in the medical laboratory community and able to providing a reliable scientific basis, can be legitimate and a logical starting point. Nevertheless, the truth is that until today no consensus on this particular question resulted in any clear recommendations, approved guidelines or widely disclosed tables with these specifications.

Hence, it will be necessary, for the medical laboratory's scientific community, to work upon the different questions, challenges and opportunities that the MU estimate brings to the laboratories. To adopt a concept that aims to contemplate all the several variation sources, or at least their effects on the patients' results, those different uncertainty sources have to be considered when setting quality goals and specifications.

The proposal of Haeckel, *et al.*, can be a step forward, as even if the presented approach does not include pre-analytical variability, it states that "uncertainties of the pre-examination phase and/or the post-examination phase can be easily added if the corresponding data are available and add relevant information to the requesting party" [110].

This stand as essential on the process for a wide implementation of the MU concept in medical laboratories, since "unless there is clear rationale for defining goals or targets for acceptable measurement uncertainty, laboratories may not recognize whether the observed uncertainty is good or bad and whether improvement is needed or not" [57].

When directly comparing TE, MU-A and MU-B, it is observed that the results for MU-A consistently showed lower percentages and consequently tighter error associated / intervals of values reasonably attributable to a patient result. As for MU-B, three different situations can be verified. For total cholesterol the values are superior to MU-B, but inferior to the ones from TE. For creatinine the MU-B values are the lowest between the three formulae. On the other hand, they are the highest, regarding the glucose determinations. The case for creatinine is interesting, because it can be seen bias in that situation. For the glucose measurement it can be attributed the main influence on the result to the high value of the pre-analytical uncertainty, confirming the pre-analytical phase as one of the sources of uncertainty with potential impact on the final values of uncertainty and on the, consequently, on the potential variation of patients results.

By analysing Table 6-9, it is easy to identify some similar outcome values between the results for TE and Model-A Uncertainty (MU-A), as it was expected attending to the data used on both calculations. Regarding the results for the Model-B formula for measurement uncertainty (MU-B), it was observed some variation that ended up making the difference when comparing the results with the goals of the mentioned international tables. Those differences can be attributed to the variables being considered in each equation, taking into account the added data of pre-analytical uncertainty and of the uncertainty of the calibrator assigned value, considered in MU-B, and the absence of the bias value for each measurement, obtained from the EQAS results for the other equations, which was simultaneously disregarded also in MU-B.

So, they have a formula for MU (Model-A) which resembles the TE calculation, using the exact same data, only varying the mathematical basis for combining that data. Thus, this first formula does not introduce any new concepts to the usual evaluation performed for TE, it just applies the rules for uncertainty combination/propagation to the same data. Submitting these values to the appreciation under the TEa limits/guidelines, it can be observed the same performance for all the analytes, which could be expectable, according to the premises supporting both. Concerning the Model-B formula, it introduces something new, adding the variability/uncertainty associated with the pre-metrological procedures, as well as the uncertainty assigned to the calibration material.

The pre-analytical phase is the step of laboratory procedures that most contributes as a source of errors; this reality is well known and recognised [112]. Others studies state that extra-analytical phases can account up to 93% of the errors within the entire diagnostic process, pointing out more than 80% attributable to the pre-analytical phase [113], reinforcing this stage as an important source of concern and potentially variability/uncertainty. This variability can be considered as pre-metrological uncertainty, “beginning when the needle is first inserted into the vein and lasts until the sample enters the measurement system” [75]. This way, there are several sources of error in the

pre-analytical phase that can contribute to erroneous results [90, 113-116], which directly adds variability to the final result and can in some cases be the dominant font of uncertainty [75, 86] . Anyhow, errors such as missing identification, wrong sampling tubes or ordered tests are not accountable and should not be confused with the quantifiable pre-metrological uncertainty described [79]. In the last years, several studies have been providing records and useful data on this subject, proving these facts and demonstrating how these gross errors should not be accounted for. [79, 84-87].

These premises, as well as the observed weight and influence on the final uncertainty value due to the pre-analytical factors variation (U_{pre}), support the necessity of further studies in this specific uncertainty component. This should be a reality for all laboratories, as these variations can manifest in response to the conditions, materials and procedures of each laboratory. The input values considered should therefore reflect the intrinsic variation of those same laboratories, which directly affects the results of the analysis of their patients' samples. Bearing this in mind, the next step on this work was exactly to promote kind of study, thereby obtaining such data and information, in order to be able to substitute the used literature values, incorporating actual data regarding Gnóstica's day-today work and its influences concerning the pre-analytical phase and variables.

7.3 Pre-analytical Uncertainty

Historically, the focus on improvement of the laboratory quality management has been directed to the analytical phase. Still, in the medical laboratory community, it is widely recognised that the pre-analytical phase is where most of the laboratory errors occur during the total testing process (TTP), accounting for approximately 60% to 70% of all the occurrences affecting the testing process or even the test final result [88-91]. Sources of pre-analytical variability can be identified through the different procedures during this phase, starting with patient's preparation but expanding in the sample collection, transportation, processing and storage [90, 91, 113, 115, 116].

Even the time required for the pre-analytical phase is often underestimated, being appraised that "it takes up more than 58% of total laboratory time required", with the "actual analytical phase only taking up around 25% of the time" [116].

Being the pre-analytical phase "recognised as the most vulnerable part of the total testing process" and given its "impact on the quality of results of laboratory testing, pre-analytical errors have been included within the greatest challenges to the laboratory professionals" [91, 117].

Although it is extensive the literature on the subject, with different standards issued, per example, by the Clinical Laboratory Standards Institute (CLSI), with guidelines regarding the collection of blood specimens or the handling, processing and the transport of samples, the "management of unsuitable specimens and reporting policies are not fully standardised, nor harmonised worldwide" and "the compliance to those guidelines is low" [117], just as "venous blood sample collection guidelines are not always followed [118].

In a recent study developed in medical laboratories in the UK, on pre-analytical errors monitoring and reporting, it was observed 30% of the laboratories do not routinely monitor the pre-analytical phase. Another finding was that there are

only two quality indicators from this particular phase which are recorded by greater than 50% of laboratories, being “mislabelled samples and booking-in errors”. Also, stated as “most surprising” was the fact that “markers of sample quality such as haemolysis, which is well documented to interfere in test results, are monitored by less than 50% of laboratories” [103].

These facts take particular relevance considering UK’s position as a reference in good laboratorial practices, being inclusively one of the few countries in Europe with 50% or more of medical laboratories already accredited, rising to 80% if it refers only to clinical biochemistry laboratories [13].

The IFCC WG-LEPS, in an attempt to reduce errors in laboratory testing, developed a project aiming to create a common system for the monitoring and reporting of quality indicators incorporating the main TTP phases. In a publication with some preliminary data of the project, in what regards the pre-analytical phase and considering the volunteer participating laboratories responses, revealed an high percentage of errors, suggesting to the authors the lack of appropriate documentation for defining criteria and operative procedures or the lack of its proper implementation by the laboratory staff [104].

So, even though it is often assumed that pre-analytical variation, or the uncertainty associated to this phase, can be considered negligible if all the sources of variation are standardised, the facts above cannot be disregarded. Moreover, the results from previous studies, which demonstrate that the pre-analytical variation is not negligible for many analytes, support pre-analytical variation as a factor that should be taken into account when estimating the measurement uncertainty in the medical laboratory [84-86].

The most frequent mistakes registered in the pre-analytical phase are related with the incorrect/insufficient tube filling, the usage of inappropriate containers and patient identification or request procedure errors. Concerning the influence or the effects of these errors in patients’ outcomes, it is estimated that approximately 25% of the occurrences are considered to have direct influence

in the test results or the patients' healthcare procedures, with most of the cases ending up resulting in test repetitions [90].

Being, as stated, pre-analytical variation sources considered various and diverse, "ranging from biological and physiological events to technical details of specimen collection and transport" [84], the major variables involve patient preparation, sample collection and handling, as well as sample transportation, processing and storage [91]. Sylte, *et al.* also states that the uncertainty chain starts with the preparation and choice of blood-collection tube, in a continuum through "variations in variables that include filling volume, sample mixing, clotting time, extent of haemolysis, centrifugal force, room temperature and humidity, and amount of time before analysis". The authors end up concluding that "even with optimal routine, some variation is inevitable" [86].

It is know that, directly regarding the analytes in study, the factors that can influence the most in any potential result variation are icteric, haemolysed or lipemic samples, mostly in the case of creatinine; cellular glycolytic activity, this particularly in the case of glucose, which can decrease up to 5% only in the first hour; or factors like the patient's posture and the prolonged use of the tourniquet during the venipuncture, especially in the case of total cholesterol [115, 119, 120] .

These variation/uncertainty sources, for individual samples, are systematic in their nature. Nonetheless, on daily laboratory work these errors can be considered as random and its variations determined to estimate the pre-analytical component, allowing later to account for them in the combined uncertainty estimate, associated to patients' results [85, 87, 95].

In practice, when studying these variables looking for an estimate of the laboratories pre-analytical variability, it is necessary to adapt the methodologies to the procedures and resources of the laboratory in question, integrating the experiment as a part of the normal laboratory processes [84].

As the objective was to determine Gnóstica's specific pre-analytical uncertainty, its specific practice and procedures were applied, this without comparing or addressing the using of different types of collecting tubes, collecting and handling procedures or centrifugation conditions. This allowed, on every puncture and blood withdrawal taken, simulating just another sample collection and processing, considering and estimating the variability from the procedure and due to processing deviations that can occur during the normal daily work flow, within the laboratory's own and normal routines. Afterwards, the sources of variation/uncertainty were individually studied as blood collection, sample storage, sample processing and regional transportation.

So, the aim of this study was to quantify the uncertainty associated to a clinical laboratory result, arising from the pre-analytical variation, on the specified analytes: creatinine, glucose and total cholesterol.

As for the pre-analytical factors and their influence on the analytical outcomes, it was demonstrated, for the analytes tested, that venipuncture represented a real source of variation, being the only variable common to all the three quantities. Glucose, as expected, showed to be sensitive to sample processing delays, as well as to regional transportation. Sample storage and different storage conditions seemed to affect more the other two analytes, particularly in the case of creatinine testing. In the mentioned situations, the obtained coefficients of variation were higher than the analytical imprecision, CV_A (%), corresponding to the laboratory's intermediate precision.

The results obtained were consistent with those from other similar studies, to the same analytes, where Fuentes-Arderiu, *et al.* [85] and Sylte, *et al.* [86] reported, respectively, coefficients of variation of 2,3% and 1,5% for Creatinine; 3,2% and 4.0% for Glucose and 1,2% and 1,6% for Total Cholesterol. Also Kouri, et al. [84] estimated a pre-analytical variation for Total Cholesterol to be 0.7%. These values that can be compared to the ones from the present study, excluding sample storage or regional transportation (Creatinine: 1,15%; Glucose: 3,87%; Total Cholesterol: 0,29%).

These last variables referred, and the associated data, although not included in the final calculations of combined uncertainties, are presented both to demonstrate its potential variation effects and so that they can be taken into account considering the presence or absence of those particular procedures in the laboratory's routines. Different laboratories, or samples with different "origins" but tested within the same laboratory, will have different uncertainty chains associated, which should be considered regarding the laboratory's reality in terms of its daily work routines, samples collection and processing.

Recently Fuentes-Arderiu stated that "when measuring a sample in any moment of the stability storage conditions, a new stability-specific measurement uncertainty appears". According to the author "this measurement uncertainty should be added to the uncertainty budget of the measurement under consideration", which supports the need for the laboratories to undertake such studies, as he also advocates [121]. Supporting this statement, Kutasz, et al., considering temperature and storage time as major factors affecting the stability of clinical samples, stand by the need and possibility of laboratories assessing these variables, depending on its own day-to-day realities. Remembering situations of "determinations added to a stored sample" or "patients distributed over large geographical areas", the authors agree with the mentioned proposal of Fuentes-Arderiu, affirming that such result's MU should reflect the clinical sample's stability, as all sources of variation should be considered and those can vary according to the samples treatment and the quantity studied [122].

In Gnóstica sample storage may be a possibility, over weekends or on tests not performed on a day-to-day basis. Also regional transportation might be an important factor, bearing in mind the existence of multiple and external collection sites in the Algarve region. So, if not negligible, this data should be included in the calculations of the combined uncertainty of samples subject to these particular conditions.

In compliance with previous studies and publications [79, 84-87, 94, 95], it was demonstrated that the pre-analytical phase should always be considered as a

potential source of variability and uncertainty, as demonstrated in previous studies and publications. Thus, the relative uncertainty associated to the pre-analytical phase should not be considered negligible and its value should be included when estimating the uncertainty of measurement results on patients samples, in medical laboratories, as other authors have previously done or defended [32, 59-61, 75]. These considerations are also supported in a position paper by the European Diagnostic Manufacturers Association (EDMA) regarding the estimation of uncertainty of measurement in medical laboratories and the uncertainty sources to consider [92].

It is so recommended that laboratories develop studies if this nature, allowing not only to estimate their own pre-analytical uncertainty, which is associated to the results of their patients, but also to identify the sources of variation with the most influence in the and weight on the final result. This is one of the great potential outcomes of the uncertainty determination, which empowers the laboratories with the capacity to act and to improve, once identified the weaknesses on chain of procedures of their own routines.

Thus, it is essential that each laboratory's particular circumstances are considered, taking into account the processes of sample collection, its materials, procedures and conditions, as of sample storage and regional transportation, so that each potential variation source it can be assessed in detail, and its individual contribution to the combined pre-analytical uncertainty can be reflected.

Along with the implementation of quality guidelines and recommendations, to improve quality and minimize pre-analytical uncertainty it is mandatory to raise awareness at the laboratory's management and staff levels, to locally, on site, know, study and identify the various variation factors, their influence and how to deal with each one. Several authors have defended that "to reduce random pre-analytical variation it is vital to identify the contributions of the various uncertainty sources, from venipuncture to analysis" [86]. Also that "more quantitative information is required in this field, especially to learn if the pre-

metrological variance varies with the value of the measured quantity (heteroscedasticity)" [85]. Being particularly important the necessity to agree upon the factors to be included and excluded in the estimates, considering the extent of laboratory services, such as regional responsibility. "The estimates could be tabulated, for example, for fasting morning samples, afternoon samples and regional transportation" [84].

A positive point is that laboratories are alert to the present situation, being aware and willing to "invest" in the pre-analytical phase quality improvement. Cornes, et al., in its recent study, entitled "*Monitoring and reporting of pre-analytical errors in laboratory medicine: the UK situation*", reported that "of the laboratories surveyed, 95.9% expressed an interest in guidance on recording pre-analytical error and 91.8% expressed interest in an external quality assurance scheme" [103], which is definitely a significant resource and an important tool to implement, allowing benchmarking between laboratories and enhancing any potential improvement opportunities. So we have to agree that "managing the extra-laboratory phase of the total testing cycle is the next challenge for laboratory medicine" [105], laying up as a critical milestone in order to improve patient safety and on the road to total quality management.

7.4 Measurement Uncertainty in the Medical Laboratory (II)

7.4.1 New application of the different MU estimate formulae - Gnóstica´s Pre-analytical Uncertainty (*2013 Update*)

After estimating the pre-analytical uncertainty specifically associated to Gnóstica Medical Laboratory, its patients' samples and test results, it was then possible to substitute on the MU-B formula (5-2) the data from the literature, previously used to account for this uncertainty component in the total measurement uncertainty estimate.

The aim of that particular study was set to deliver a more realistic value of the final global uncertainty. In the end, applying those results to the MU-B formula allows to present the best uncertainty estimate, with that being the one that best describes or characterizes each measurement and the dispersion of values that can probably be attributed to a particular measurand, at Gnóstica Medical Laboratory.

It also demonstrated the necessity to consider the pre-analytical phase variation as an assumed uncertainty component, not only confirming it as a source of variation, which was already widely recognized and documented, but validating its measurability and above it all its potential major influence in an uncertainty estimate, consequently holding a potential of influence on patient's results.

This analysis could be operated at two levels, by directly comparing the outcomes from the 2011 data, on Table 6-9, with the 2013 results, on Table 6-16, and also by considering the individual influence of this particular uncertainty source, on each analyte, analysing Table 6-16, considering the data on Table 6-15.

So, in the first case, observing and comparing the variation of the pre-analytical uncertainty from the literature (creatinine: 2,3%; glucose: 3,2%; total cholesterol: 1,2%), and the results of the study developed at Gnóstica (respectively: 1,15%; 3,87%; 0,29%), becomes evident that this pre-analytical influence is differentiated, depending on the analyte, but also that it varies accordingly to the laboratory conditions and procedures, justifying the realization of in-house specific studies by different laboratories, in order to assess and quantify the pre-analytical uncertainty associated to each laboratory's results, in compliance with other authors findings and statements [121, 122].

In general, when comparing the uncertainties estimate (MU-B: *year 2011 and year 2013*) it can be verified the increase or decrease in the measurement uncertainty final value, reflecting the same way change in the pre-analytical standard uncertainty, for each analyte. This indicates, once again, that the uncertainty estimate not only can be affected by the pre-analytical component, but also and most importantly, as stated above, that this uncertainty source can be variable from laboratory to laboratory, considering the results from the developed study, in view of the previous data used.

Regarding the within analyte variation, when comparing the different formulae and considering the latest results (*year 2013*), three situations can be observed. What is first noticed is the high value of pre-analytical uncertainty affecting the results of glucose's measurements. This variation source assumes preponderant weight on the estimate, increasing the value of the MU-B outcomes, when comparing to the other MU formula, or with the TE calculation. Total cholesterol, having the lowest pre-analytical uncertainty, when this value is combined with the calibrator uncertainty and with imprecision, for the MU-B estimate, the final results become very close for the different estimates, as there are not considerable differences regarding the measurement bias value used and the previous mentioned uncertainty components, capable of affecting or differentiating the final estimates.

On the creatinine's results, concerning the MU-B model formula, where the data from the calibrator uncertainty and the pre-analytical uncertainty are lower than the bias value, it is observed the decrease in the final result of the measurement uncertainty. However, it is observed a considerable difference between bias values for the two auto-analysers, which is reflected later on the final uncertainty values, when comparing the different applied formulae.

This fact reveals the importance of bias studies, supporting its usefulness in the medical laboratory. In order to reduce, minimize or eliminate bias, it is important to study it, acknowledging its existence and measure it. This should be done, if not in other way, by means of the EQAS results or other proficiency testing studies. These are part of the practical and technical requisites of the ISO15189 standard, for the medical laboratories' quality management systems and records, in the validation processes of the implemented measurement methods which are submitted to the accreditation process by the mentioned standard.

Regarding the use of the bias or of the uncertainty assigned to the calibrators in the different MU estimates, the centre of the question is traceability, being a vital tool to ensure trueness having a calibration hierarchy traceable to a high reference measurement procedure. Values assigned to calibrators should be metrologically traceable as far up a calibration hierarchy as possible, so that end results are also traceable and thereby can reliably provide for trueness and comparability to the measurement [27, 65]. Assuming that the trueness control provided by the manufacturers represents the most appropriate approach for the quantitative expression of that performance characteristic, where a traceable calibrator is used, the analytical bias should not introduce uncertainty to the testing system [33, 73], being the calibrator's uncertainty, if presenting high order traceability, the element of choice to the trueness input value.

Nevertheless, EQAS results are used in medical laboratories procedures aiming to detect, accordingly to the established performance goals, deviations to those desirable or minimum quality requirements. This is usually performed considering the Total Error, which has defined specifications for allowable total error, but can also be accomplished for other performance characteristics, as imprecision or bias.

Consequently, in this study, the bias was assessed considering the mentioned measurement validating procedures under the scope of the accreditation by the ISO 15189 standard. So, the bias values were compared with the international quality specifications tables from Ricos, *et al.[17],* which contain the desirable specifications for bias, along with the specifications for imprecision and allowable total error, with these last ones being presented in Table 6-16. The results could, also be compared with other tables, if and whenever necessary for assessment, as already discussed in this work.

In the previously mentioned situation, for the creatinine measurement, although it was identified a considerable difference between the determined bias values for the two used equipment, this specifications were not violated. Specifically in this case, for which was obtained a maximum bias of 3,22%, being at 4% the desirable specification for bias for serum creatinine, on the “Desirable Specifications for Total Error, Imprecision and Bias Table”, by Ricos, *et al.* [17]. Note that the same final considerations can be made for the other two analytes, since all fulfilled these quality goals.

If it had been verified the occurrence of any failure to comply within these quality performance assessments, that would have been shown in the EQAS results, and had then to be subjected to reflection and evaluation by the laboratory’s Quality Management Department, being subsequently submitted to the appropriate procedures to eliminate or minimize the confirmed occurrence. Subsequently, the bias, and its uncertainty should be assessed for significance, being considered its eventual inclusion on the uncertainty estimate, even with

the traceable calibrators and their associated uncertainties being already accounted for in the estimate.

The added-value, and one of the objectives, of introducing in a laboratory's routine and measurement procedures high standard calibrators, traceable to reference materials and methods, with uncertainties associated to their assigned values, is exactly the reduction of bias to residual or negligible values, seeking to increase the results trueness and to ensure the comparability of those measurements. Nevertheless, even though one of the basic concepts behind the implementation of uncertainty measurement in the medical laboratory is that the measurement procedures should be, in principle, unbiased [27, 72], when a variation affecting that same measurement is detected at this level, revealing possible implications regarding the measurement's trueness, it should be assessed and considered for significance.

As discussed above, even in methods with traceable calibrators are possible to occur, being detected, problems or irregular variations regarding measurement bias, on the other hand, methods without traceable calibrators are expected and most likely to being affected by these situations, carrying verified significant bias. It is possible to know and account for that bias and its uncertainty by assessing it directly using certified reference materials (CRM). Alternatively it is also possible to do so by participating in proficiency tests or by the results of EQAS. Subsequently, different approaches for the estimate can be found [31, 35, 99], all having in common the understanding that when calculating the uncertainty of bias it comprises the uncertainty of the bias itself, regarding the measurements for its estimate, but also the uncertainty regarding the reference value or reference material used in the assessment [123]. So, the bias value cannot be exactly known, as it always encompasses the uncertainty of the certified reference materials used and/or the unavoidable imprecision of the routine procedures used to obtain the replicate measurements or the mean value for the reference material [25].

Notwithstanding, to be in compliance with the GUM principles, any known bias should be reduced or minimized by re-calibration or by the use of a correction factor, being this way corrected or even eliminated. But, if this principle generally acknowledged, it is also of unanimous comprehension that a correction of the bias carries itself an uncertainty that has to be considered whenever that same correction is performed [25, 123].

Anyway, the known bias and its uncertainty, or the uncertainty associated to the bias correction, should only be accounted as an uncertainty source in a measurement uncertainty estimate if considered significant. For that matters, different approaches have been proposed, being evaluated the significance of the bias, facing its uncertainty value, by a statistical *t*-test result [31, 73], or it may even be considered a more empirical and decision rule, with laboratories considering "to ignore uncertainty of bias values it they are less than an arbitrary cut-off of 20-30% of the intermediate imprecision" [25].

Considering this problematic, different analysis and reviews can be found on the literature on the bias subject, being each more or less extensive, such as Linsinger's, T. P. [123], or more recently from Theodorsson, *et al.* [124].The authors usually address bias and its causes, the general ones and the measurement specific, considering the method itself, its interferences, the matrix effects and the commutability of the materials used. Are also approached the bias estimate and its own associated uncertainty, as well as the potential correction, or not, of the values detected, considering about possible restrictions and impediments to do so. The conclusions point the reference measurement methods and commutable reference materials as the basis of all efforts for minimizing or eliminating bias yet considering about the bias significance and its possible use and inclusion in measurement uncertainties estimates [123, 124].

As stated by Theodorsson, *et al.*, hitherto there is no consensus or clear guidance on how to account for observed uncorrected bias as an uncertainty component in the uncertainty estimation. So, several different approaches and

models have been proposed as alternatives to include bias in measurement uncertainty estimation [125-127].

Ultimately, as mentioned above, considering that where a traceable calibrator is used, analytical bias should not introduce uncertainty to the testing system [33, 73], since the manufacturers specifications for the calibrating materials used in this study stated its traceability to the Reference Measurement Procedure (Isotope Dilution Mass Spectrometry - IDMS) and assuming that the presented uncertainties associated to each calibrator provided the trueness component for the measurement, were considered to be fulfilled the necessary conditions to adequately implement MU-B model (5-2).

Thus, based on the specifications of the manufacturers method of calibration, joining the fact that the EQAS for these analytes did not indicate any bias problem [31, 82, 96], the bias could be assumed negligible.

Finally, the uncertainties associated to the calibrators assigned values could be used, as mentioned, as the trueness components in the MU estimates, being considered along with each analyte pre-analytical uncertainty, as well as the specific imprecisions for each one, enabling the MU-B model formula not only to be properly implemented as to be considerable suitable for the designated purpose.

7.5 IPAC's Accreditation Auditing

The main reason for this collaboration and for Gnóstica's investment in this project was finally put to test at this phase.

Although having now a mandatory requirement regarding measurement uncertainties, neither the accreditation standard nor the IPAC specify an estimating model or formula to implement. As already addressed, not the GUM or other publications with particular focus on the medical laboratory, even when outlining the principles and possible approaches to this estimates, deliver a definitive and consensual approach to follow [98, 128].

Consequently, having no particular model suggested as preferential, the IPAC only requires that the applied approach proves to be technically valid and applicable to the methods in question, following the ISO 15189 Standard statement "as the laboratory shall define".

So, like was stated before, this first contact between the auditing teams and the accredited laboratories, following the new version on the implemented standard, represents also a moment of learning and discussion among peers.

Besides the inexistence of a defined or restrict model implemented for the MU estimate, also there are no published performance goals specifically for the outcomes of this quality tool, as addressed on section 7.2. For this reason, are again presented the specifications on Ricos, *et al.* and on the CLIA's tables, which were used as point of reference.

To this particular, all the analytes, on the outcomes for each formulae applied, fulfil the quality requirements presented, being the ones on Ricos's Table, or the broader ones on the CLIA's Table, considering that either one is accepted for setting the minimum quality specifications.

Considered a normal procedure in these processes, the auditing report identified different situations, marked as Non-Conformities (NC), which required the analysis, correction and reporting back, by Gnóstica's Quality Department.

Being this a maintenance auditing, as Gnóstica was seeking to keep the Accreditation Certificate that already had, as opposite to being a new candidate to the accreditation process, the laboratory got from one to three months to respond to the identified situations, depending on being considered "*Major*" or "*Minor*" NCs.

Among the different NCs identified, were also the required MU estimates. The reason for that was that were only presented estimates for three of the 96 analytes and measurements under the accreditation scope.

Thus, the laboratory was asked give response to this particular requisite, showing "*the Combined and Expanded MU estimate for all the quantitative methods under the accreditation scope, with evidence of considering the laboratory's intermediate precision (imprecision) in those estimates*". This particular excerpt of the auditing report is presented in Appendix M.

For replying to IPAC, the formulae were directly applied to the remaining analytes and measurements, discriminating the uncertainty sources being considered. The principles supporting the approaches were also included in the technical report, having the laboratory assumed the objective of including the values of uncertainty associated to the measurements calibrators in the future MU estimates, as well as data from other uncertainty sources, namely the pre-analytical uncertainty. In compliance, the laboratory is already gathering the necessary data, asking the manufacturers of the measurements and methods involved to provide for information and uncertainty certificates for those analytes. Also, regarding the pre-analytical uncertainty, following the work developed in section 5.3 and applying the same methodology, the study was already implemented to sixteen other analytes, as shown in Appendix K and Appendix L, being the intention to proceed with the studies, by assessing the other remaining analytes.

While aiming to, in the future, being able to define proper performance goals to the MU estimates, in Gnóstica's response to the auditing report was also stated that, considering the absence of specific MU desirable limits and publications on the matter, in this first phase, and in parallel, the laboratory will continue calculating the Total Error.

Although having already received positive feedback from the IPAC regarding the response to the preliminary auditing report, including the information sent to give answer to the NC about the measurement uncertainties estimate, validating the data sent and confirming the renewal of the accreditation, a new certificate will only be emitted posteriorly. With the approval to the responses sent in the sequence of the auditing report, the accreditation certificate, issued in the end of 2015 (Appendix G) remains valid, until the emission of a new one, which will to replace and update the previous.

7.6 Measurement Uncertainty in the Medical Laboratory (III)

This section presents the final application of the MU formula, realized after the preliminary testing performed until now. After the studies with the laboratory's data regarding the years 2011 and 2013, in this final phase of the project, with updated data from 2015, the last and improved version of the MU formula was applied.

Considering the main objective of reaching a formula able to respond to Gnóstica's necessities regarding the accreditation requirements and procedures, imposed by the ISO 15189 standard and applied according to IPAC's auditing rules and criteria, the provisional results obtained from the implemented methodologies from sections 5.2, 5.4 and particularly section 5.5, were considered and discussed with the laboratory's Technical Board.

Revising the initial approaches' outcomes allowed to finally achieving a formula that fulfilled that primary objective, being essential the experience, criticisms and posterior acceptance, or validation, of this project's work and of its results', attained after, and from overcoming, the experience with the IPAC's auditing. Being able to obtain, from an external review, commentaries on the work developed, with a feedback assuming, with recognized expertise, that model and approach presented as being valid and applicable in the laboratory's routine was the best confirmation of the potential success of this project.

With the previously mentioned update on its application, this final formula and its outcomes were considered to be representative of the measurement process and the best MU estimate for the results at Gnóstica Medical Laboratory. Thereby, the equation was lastly applied to the laboratory's data, for a final assessment, being the necessary data collected for the calculations presented in Table 6-17. The relative standard uncertainties to include in the estimate, as well as the obtained MU Combined Uncertainty and the final MU Expanded Uncertainties, for each studied analyte, are the presented in Table 6-18.

7.6.1 Gnóstica's MU Estimate - Final Formula (MU-B)

The studies previously developed, presented on the early sections, resulted in consecutive updates to the model being implemented. First, they enabled to integrate the values of Gnóstica's own pre-analytical uncertainty on the laboratory's MU estimates, regarding the measurements methods under the scope of the project. Later, after analysis, the final formula was optimised to incorporate, in only one estimate, the measurement uncertainty for a broad range of values, combining the high and low levels of the measurement's intermediate precision.

Furthermore, was decided to use the uncertainty associated to the calibrator, considering it to be the most reliable data representing the trueness of the respective measurement, assuring its traceability and comparability, also objectives of the implementation of measurement uncertainty estimates in the medical laboratory. This decision takes support in the actions of the EU Directive 98/79EC, seconded by the ISO Standards 17511 and 18153, obligating the IVD manufacturers to assure and demonstrate the metrological traceability of their products and to make available the uncertainties associated to the calibrators assigned values, as it was addressed on section 2.

Also as mentioned before, bias, as a systematic source of variation, is to be properly handled, reduced and minimized or eliminated, being this to one of the basis of MU. Thus, as long as adequately assessed and controlled, considering its recommended desirable specifications, preferably through internal studies using certified reference materials or by the means of external evaluations, like EQAS, it should not be added to the estimate. So, considering the available certificates from the manufacturers, regarding the methods' calibrators in the study, trueness and traceability were found to be assured, and the values of the calibrators' uncertainties were included in the estimates, accounting for that performance characteristic.

Looking to adapt the final MU formula to a broader application, combining the imprecision data from the different levels of the IQC allowed achieving a MU value that can be applied to the whole range of results in a measurement, characterizing the dispersion of values in different dimensions of results, with only one estimate.

Although it possibly requires further studies, to validate the procedure internally at Gnóstica, this experimental combination of the different levels of imprecision, incorporating in the MU estimate a pooled imprecision, as proposed elsewhere [99, 101, 102, 129, 130], covering the low and high range of the measurement was considered positive, revealing adequate outcomes, comparable to the previous. Anyway, this should take consideration on the possibility of different variations along a wide range of measurement, as a described heteroscedasticity phenomenon [58], what could justify different MU estimates at the different control levels, for different concentrations of the measurand [37, 130].

Something similar to the pooled imprecision could be attempted to do with the equipment. However, just observing the imprecision values for the creatinine measurement it is obvious the necessity of doing a dedicated study to evaluate the possibility, considering for any significant differences between the equipment. This was performed by Magnusson, B., *et al.*, in a study where the authors assured similar variation within and between seven different equipment assuming the bias to be negligible, compared to the precision [37].

As already addressed, regarding the performance specifications and the MU goals, has been considered that an approach based on biological variation may be applied to compare and evaluate measurement uncertainty outcomes [98, 99, 111], this approach should actually be preferred as a basis for establishing the desirable or minimum quality goals, specifically for MU, "*because of its transparency and scientific base*", over other different approaches [110].

The new RiliBaek, the “Guidelines of the German Medical Association on quality assurance in medical laboratory testing”, integrating new quality permissible deviations, determined based on a root mean square deviation (%RMSD) calculation, and so, expressing these performance goals in an approach similar to the concepts of uncertainty estimates, present minimum specification performances that can be also be considered when intending to compare and evaluate the laboratory’s MU estimates outcomes [131].

As addressed before, on section 7.2, the proposed “Permissible limits for uncertainty of measurement in laboratory medicine”, by Haeckel et al., seems to have given a consistent step towards the creation of new and adapted performance specifications. This proposal also claims the possibility, hereafter and given its relevancy, of accounting for different sources or variation, like pre-analytical uncertainty, which is not being considered presently [110].

Relating this subject to the discussed above, regarding the pooled imprecision, these Tables present specifications for a range of values, fact that helps to label this as a suitable procedure when estimating measurement uncertainties. For that matters, Haeckel’s Table and the RiliBaek’s Guidelines presents similar permissible limits, according to which, comparing the final results here obtained, in section 6.6, the MU estimate generally complies with the specifications, being observed punctual borderline violations in the creatinine and glucose estimates, but only in Haeckel’s Table (Permissible U%: Creatinine=9.55; Glucose=8.25; MU Estimate (%): Creatinine=9.6; Glucose=8.34) [110].

These violations are observed in the analytes with the higher associated pre-analytical uncertainty, which has to be remembered as not being contemplated in these performance specifications, fact that can be considered as supporting and justifying the necessity for the establishment of new and consensual guidelines, adapted and updated to the concept and tool being introduced in the medical laboratory. This is reflected in Westgard, J.O. statement, affirming that: “*unless there is a clear rationale for defining goals or targets for acceptable*

measurement uncertainty, laboratories may not recognize whether the observed uncertainty is good or bad and whether improvement is needed or not" [57].

7.6.2 Standard Uncertainties' Index

Lastly, determining the different relative uncertainties index, regarding the final combined uncertainty, allows identifying the components differentiate contributions to the MU final value. This, consequently, reveals which have the most potential of causing any variation on the final measurements results, but also indicates on what component the laboratory should concentrate efforts to reduce its variation. Thus, this evaluation provides important information, revealing the measurements' probable weak points, regarding the causes or origins of the variability of its results. This is one of the possible benefits, if not an objective, of implementing a protocol for estimating measurement uncertainties.

From the presented results can be observed that variations between 10-20% regarding the calibrator's uncertainty, being this the component that consistently less contributes to the global uncertainty. As expected, the most evident source of variation relies on the methods intermediate precision, by the values of the different measurements imprecision, presenting values never lower than 25%, going up to 71%. The exception here, representing the bottom margin for the imprecision levels, is the glucose measurement, having its pre-analytical uncertainty assuming as dominant source of uncertainty, with 67% of the total of the combined uncertainty, in a component that reveals to be as low as 11%, in the case of total cholesterol testing.

This exercise serves as a graphical demonstration, which illustrates a situation that probably as reflection on many different analytes and on their measurements, where the predominant source of uncertainty is the method's intermediate imprecision, justifying all the procedures and attention that these testing phase gathers in the laboratory. Other analytes will probably present high values of pre-analytical uncertainty to, being, as demonstrated in this study, a variation source to consider and that properly be valued, in order to improve its performance characteristics, not over-increase the MU estimates.

7.7 MU Estimate Final Tool – Gnostica's working spreadsheets

As mentioned above, in section 6.7 is presented the MU estimating tool, developed for the implementation of these concept and procedures at Gnóstica Medical Laboratory.

The principles and methodology applied have had the approval of the Laboratory's Quality Management Department, having its implementation, practical applicability and MU outcomes also been validated, on the sequence of the procedures, and reports, regarding the renewal of Gnóstica's Accreditation Certificate, as well as the feedback on the last Accreditation Auditing.

The implementation of the presented tool is meant to be simple and intuitive, using data and terminologies applied in the everyday routine and already being collected within laboratory's QMS. It can be applied either restrictedly by elements of the quality department or by distributing responsibilities to other members of the laboratory technical staff, as decided and assigned by the laboratory's management.

Concerning the data introduction, the tables are also easy to replicate. The objective is to have one file for each different section of the laboratory, per assessment year. However, the application in the laboratory may be decided otherwise. Anyway, regardless of the decision, the important is to distinguish between the different working sheets of the file, which are interrelated. All the data introduced on the sheets represented in Figure 21and Figure 22, respectively the laboratory's intermediate precision (IQC) and the systematic variation data (EQAS), (for such cases where there is a significant bias being considered, as addressed on the previous sections), is automatically integrated in the Figure 23 table, for the final estimate.

Finally, the MU estimate will be obtained considering the inclusion on that final sheet of the remaining uncertainty components. Generically, as proposed on section 5.6, these last components are the Calibrator's and the Pre-analytical Uncertainty. The final MU values are obtained through the integrated application of equation (5-2), resulting in the Combined Uncertainty (u_C). The final Expanded Uncertainty (U) is obtained directly, with the spreadsheet using the u_C, previously calculated, being applied on equation (5-3), on page 55. In the situation where other uncertainty components are considered, the first step of the process, to obtain the u_C is accomplished by the use of the general equation (2-1), with the final step being the same as described before.

In order to avoid unwanted and unexpected mistakes, in every worksheet, the white columns are meant for the data input; the darker columns (grey) indicate where an outcome for each specific identified parameter will be displayed. These dark areas are blocked to the users, likewise the formulae areas, which are not shown on the above figures of the spreadsheets, not being accessible in the working area environment.

This is the corollary of this mid-term project, which is now being put to test, with Gnóstica's MU Estimates already being determined, for the current year's data, through the implementation of the working tool here presented.

8 CONCLUSION

8.1 Conclusion

Medical Laboratory Accreditation, based on ISO 15189:2012 Medical Laboratories – Requirements for quality and competence, in its technical requirements, states that the applying laboratories “shall determine measurement uncertainty for each measurement procedure”, plus it yet declares that the laboratories “shall define the performance requirements for the measurement uncertainty of each measurement procedure”.

Given this broad and flexible definition, laboratories may have the freedom to choose the methodology to implement, along with performance specifications to apply. However, National Accreditation Bodies (NABs), which usually hold these procedures, its assessment and approval, have recommended guidelines, narrowing individual creativity and promoting the technical validity, reliability and comparability of the implemented methodologies.

Although this has been verified in some countries Governments or NABs, like the National Pathology Accreditation Advisory Council (NPAAC) [96], from Australia, the South African National Accreditation System (SANAS) [97]or the Ontario Laboratory Accreditation (OLA) [130], from Canada, amongst others, the proposals are not unanimous or even consensual.

Regarding the Scientific Organizations, Clinical and Laboratory Standards Institute (CLSI) have already issued the C51-A Expression of Measurement Uncertainty in Laboratory Medicine, while the International Organization for Standardization (ISO), had a preliminary project for a Standard, that was cancelled before any publication. Currently ISO has another ongoing project, which is still under development for a Standard, ISO/NP TS 20914 Medical Laboratories – Practical guide for the estimation of MU.

In Portugal, particularly, this is a subject whit great lack of studies as of internal references and guidance. This fact may be reflected in the low search for objective implementation of accreditation systems, and consequently little knowledge and application of this valuable resource and quality tool.

Thus, this project started with the main objective of developing an approach for the estimate of measurement uncertainties in the medical laboratory, aiming to ensure a formula capable of meeting the mandatory requirements for laboratories accreditation, within the NP EN ISO 15189:2014 standard, fulfilling the necessary procedures and requisites to comply with the regulations of the Portuguese Accreditation Institute (IPAC).

In order to achieve this, different approaches and formulae for the estimate of MU were tested and compared, considering its application in the context of the medical laboratory, its outcomes and fit for purpose. In the process was given value to the inclusion of different categories and sources of variation or uncertainty, in the attempt to achieve a final model that best considered and characterised the result and the dispersion of values that can be attributed to a specific measurand

Having tested two different formulae, the Model-B formula was considered to produce very sustainable outcomes, providing reliable and realistic values of measurement uncertainty, capable of defining the procedure and its variability, well representing the dispersion of values which can reasonably be attributed to the measurand final result.

Regarding the formula itself, it is always possible to think in amendments or sought out for improvements. In fact there are other factors, sources or components of uncertainty that can be considered not being part of the categories now assessed. Sources like biological variation can be considered important to assess, for instance, when comparing two test results from the same patient or for individuals requiring consecutive monitoring, including in the uncertainty budget data corresponding to the within-subject biological variation [29, 33, 59, 76, 84, 96]. Also, have been demonstrated that influence quantities, classified as endogenous or exogenous, can possibly represent an important component of the uncertainty of a patient's result [75], being possible to adapt the formula.

Although these can reveal to be important issues, especially in the situation where the pointed potential sources of variation have effective influence, the most relevant question appears to be the reliability and traceability of the estimate. Having this guaranteed, assuring one of the main objectives of implementing MU in medical laboratories at scale, measurement uncertainty estimate has the flexibility or the capacity of combining those multiple sources, arising from laboratorial or clinical justification. Such possibility can be represented in using the general equation (8-1):

$$u_c = \sqrt{u_i^2 + u_{ii}^2 + u_{iii}^2 + u_{iv}^2} \quad \textbf{(8-1)}$$

In which will be considered the different components of uncertainty:

- i) day-to-day imprecision;
- ii) uncertainty of the calibrator assigned value;
- iii) Pre-metrological variability.

Eventually, in specific situations other uncertainty sources can be considered; e.g.:

- iv) endogenous influence quantities [75], or
- v) biological variation values [17].

Subsequently, the same coverage factor will be applied to obtain the Expanded Uncertainty, as demonstrated by equation (5-3), page 55.

The above reinforces one of the critical points, the disregard of measurement bias, under the addressed adequate conditions and as long as guaranteed the measurement traceability to reference methods and high order calibrating materials. This is assured through a specific and respected hierarchy calibration chain, with the use of certified calibrators traceable to the highest order possible. Thus, the discussion is brought to the manufacturers and to their role and responsibilities in the medical laboratory.

For these matters, ISO standards ISO17511 [42] and ISO18153 [43] followed the direction, and supported of the European Union Directive 98/79/EC [44], on In Vitro Diagnostic Medical Devices, stating that for correct medical interpretation and comparability it is essential that results reported to both patients and physicians are adequately accurate (true and precise), supporting the obligation on manufacturers to ensure traceability of their analytical systems to recognised higher-order references and to indicate the expected uncertainty of the assay calibrators. In a position paper, in 2006, the European Diagnostic Manufacturers Association (EDMA), assumed IVD manufacturers as responsible for providing information on traceability and methods imprecision, comprising the selection of reference material and methods and documentation on the uncertainty of the calibrators assigned values [92]. In compliance, clinical laboratories should take part on these responsibilities, asking for the mentioned certificates to their manufacturers and also committing to always choose and prefer the suppliers able to demonstrate and provide these requirements.

Again, the introduction and implementation of use of certified calibrating material, traceable to high order reference procedures and materials, with the uncertainties associated to the assigned values, is mandatory and vital to this process. This is the only way to aim for obtaining results traceable to those high-level calibrators and reference measurement procedures, reaching to a reliable calibration chain hierarchy that allows aiming for the desired and required trueness of the measurement procedure. The use of such materials, integrating its uncertainties in the MU estimates, allows disregarding, or to consider negligible the bias, supporting its withdrawal of the estimate.

This has been supported, being in some sense an imperative of the uncertainty concept, the bias, if known should be minimized or eliminated. Nevertheless, it is crucial to continue assessing and controlling laboratory bias, keeping the EQAS a particular and fundamental importance, since if detected do be significant, the bias value, or its correction bias, should be considered. As discussed, some options claim that, even then, bias should always be handled separately and never added to the uncertainty estimate.

Nevertheless, attending to the values presented and considering its weight in the total measurement uncertainty, these values can, and should, always be verified and re-calculated by the laboratories [129, 132-134] The same way, laboratories can and should develop studies assessing their own pre-analytical uncertainty, evaluating their specific resources and human, technical and environmental conditions, as developed on section 5.3 of this study. This particular laboratorial phase can affect the subsequent phases (analytical and post-analytical), with errors regarding sample handling or transportation be in higher level than the analytical errors [128].

In the mentioned protocol, it were demonstrated both, the potential of variation and influence of this uncertainty source, as well as the need for it to be assessed by each particular laboratory, regarding its own day-to-day working conditions and procedures. The environmental and technical conditions of each laboratory, in conjunction with the actual procedure itself, can be of great influence to these values, contributing to the final uncertainty of the results. As a recommendation, this should also be included as a requisite and part of the mandatory demonstration of quality and technical competence of a laboratory when involved in any process of certification or accreditation, along with the other quality indicators already foreseen.

Documenting, verifying and reporting data; creating quality manuals and procedures for the entire laboratory competences, accounting for all of the phases of the analytical process – specially related to sampling and samples processing, transport and storage; investing in continuous learning and training is the only way to actually achieve a reliable quality, reducing errors and the effective cost of the non-quality and improving the whole patient care system.

One important step forward is the improvement of pre-analytical EQAS. Existent pre-analytical external quality assessment programs should be having considerable investment from the medical laboratory scientific community, growing in both, scope and specificity. Once again, shared responsibilities must be taken between International Scientific Organizations, Manufacturers and the National Bodies.

According to the authors, the few programs implemented seem to substantially different, being defended a combination of the different models now in action, as “probably necessary to be able to detect and monitor the wide range of errors occurring in the pre-analytical phase”. The authors conclude that further to implement this specific programs, it should be promoted the results publication since “information of such schemes and their ability to improve pre-analytical routines in the laboratories are scarce in the literature” [135].

Simultaneously, it is necessary a strong effort towards total standardization of working methodologies, an effort that must be vertical, applied and implemented to all phases of the laboratory procedures, being submitted to constant and programed reviews and evaluation. As seen, this should, have special focus in the pre-analytical phase, by far the most neglected, despite the evidences of being in the total testing process, the one that most contributes to the laboratory error, also affecting results variation. Again, this should also comprise written procedures and protocols for the whole pre-analytical phase process; registration of all occurrences and a well-defined and strictly followed policy of acceptance/rejection of samples; supported by an intense training programs.

Recently, Kallner, A., strengthened the concept of MU, electing it to preferably "characterizing the performance of a measuring system". It was again mentioned the reduction of bias and sustained the possibility of taking pre-analytical and biological variations into account in these estimates, but, more importantly, was suggested the possibility of using computer applications to help and simplify implementing the estimates [29].

Making the connection to the principal objective of this project, these last recommendations are pointing right at the final product of this work: a tool ready to use and put to practice, meant to simplify the introduction of this concept in the daily routine of the laboratory and enabling the proper application of this quality resource.

This to say that the main goal was accomplished, as at the end of the process was obtained, as aimed to, a working spreadsheet to implement in the laboratory's own quality procedures and routines providing an instrument that is credible and fit for purpose as well as user friendly and perfectly able to be integrated as one of the QMS assessment tools, giving response to Accreditation guidelines and standards and to its requirements on evidences of quality for the laboratory's analytical process and technical competence.

This success was anticipated in sections 5.5, 6.5 and 7.5, when the model in study and development was put to test, in the first Accreditation auditing. After some clarification about the concept and the formula applied, supporting the options made regarding the sources considered, the model was praised to be fit for purpose, and so accomplish its objectives and fulfilling the requirements of ISO 15189, and the procedures of IPAC, contributing to the desired and anticipated Accreditation approval and certificate renewal.

8.2 New applications; Future Work and Recommendations

A challenge and precious goal for the near future relies on the applicability and fit for purpose, not only of the formulae and calculations of MU, but essentially of the uncertainty concept itself, and, of course, of its implementation amongst the medical community, both pathologists and clinicians/physicians, the medical laboratory staff, including technicians and finally to the patients and users of the medical laboratory services.

On this matter, questions regarding the reporting and introduction of MU estimates to the laboratory users are not consensual, with arguments forward and against, considering the opposing facts related to both clinicians/physicians and patients. If ones are already given too much information, the others are not aware of the possible variation associated to laboratory results. On the other hand taking knowledge of uncertainties associated to the results as the potential of reducing misinterpretation and probably consequently to reduce mis-diagnosis, wrong treatments or unnecessary new tests.

Other important issues regards test results deriving from calculations, like the creatinine clearance or the anion gap, or qualitative testing in the medical laboratory. The whole area of microbiology is another differentiate problem to approach in future studies. Thus, there is much to explore, and also already much being made, like in direct application and considerations regarding different the clinical situations, with several examples published elsewhere [96, 136, 137].

Also not addressed, the question of post-analytical uncertainty, concerning which other authors have considered, mainly referring the important problem regarding an appropriate number of significant figures, as reporting results, or addressing the influences in result interpretation [33, 96, 128].

As for issues that were addressed but require further studying, can be referred the combined imprecision, which was properly approached but of course requires continuity. Other similar study, as mentioned, is to verify the possibility of combining the equipment in the estimate, originating an uncertainty estimate for the laboratory. The objective and commitment in this project with Gnóstica is to continue the studies in the pre-analytical phase as well, aiming to include the remaining analytes.

Other studies are being developed, exploring the possibilities of MU applications in the medical laboratory. In a couple of examples, Ceriotti, et, al., proposed “the use of the uncertainty approach to develop an effective alarm system”, regarding the evaluation of IQC **[99]**. Jones et, al., used the uncertainty in an approach to correct laboratory results for the effects of interferences [138]. In one last example, Badrick, T. and R. C. Hawkins, “*described a novel approach to the determination of the reporting interval for an assay, one that is determined by the uncertainty of the measurement process and therefore provides useful information to the clinician about the interpretation of the result” [139] .*

From the application of the Model-B formula for MU, became clear the necessary investment from manufacturers and reference laboratories in producing and providing certified reference material, standard calibrators with high metrological traceability together with the uncertainties to their assigned values, this is one important source of uncertainty and consequently one important indicator of the result’s quality.

The objective, as addressed, should comprise the investment of all involved in the medical laboratory community, being of particular importance the effort of the manufacturers, being ambitious goals an attempt of global standardization, including the pair reference method/reference material, following the steps of the International consensus on the Worldwide Standardization of the Hemoglobin A1C Measurement. This is maybe an utopia for now, but the way to global standardization, traceability and comparability.

Another essential, it is crucial that International Scientific Organizations and Governments invest as well, in both studying and developing new guidelines and tables with new goals and limits, well defined according to the new evaluation methodologies and tools being introduced in the medical laboratory. It is obvious the necessity of actualization of the ones in use, with the incorporation of the concept and determination of uncertainty, introducing new requisites for performance and maximum allowable values. The suggested definition by each laboratory of its measurement goals or “performance requirements” does not seem to be the most indicated.

The major challenge and objective should be to follow the path towards traceability and comparability, allowing getting to a proven and accepted model of uncertainty determination, applicable to the Medical Laboratory, providing Measurement Uncertainties fit for purpose, enforcing and enhancing the way to Total Quality Management.

The combination of all the above would produce an undoubted improvement to the general and total quality and reliability of the medical laboratories procedures, performances and consequently of its results, enhancing traceability and comparability of laboratories methods’ and results’, in the end leading to more efficient and reliable laboratory services, *securing patient safety* and a better and stronger health care.

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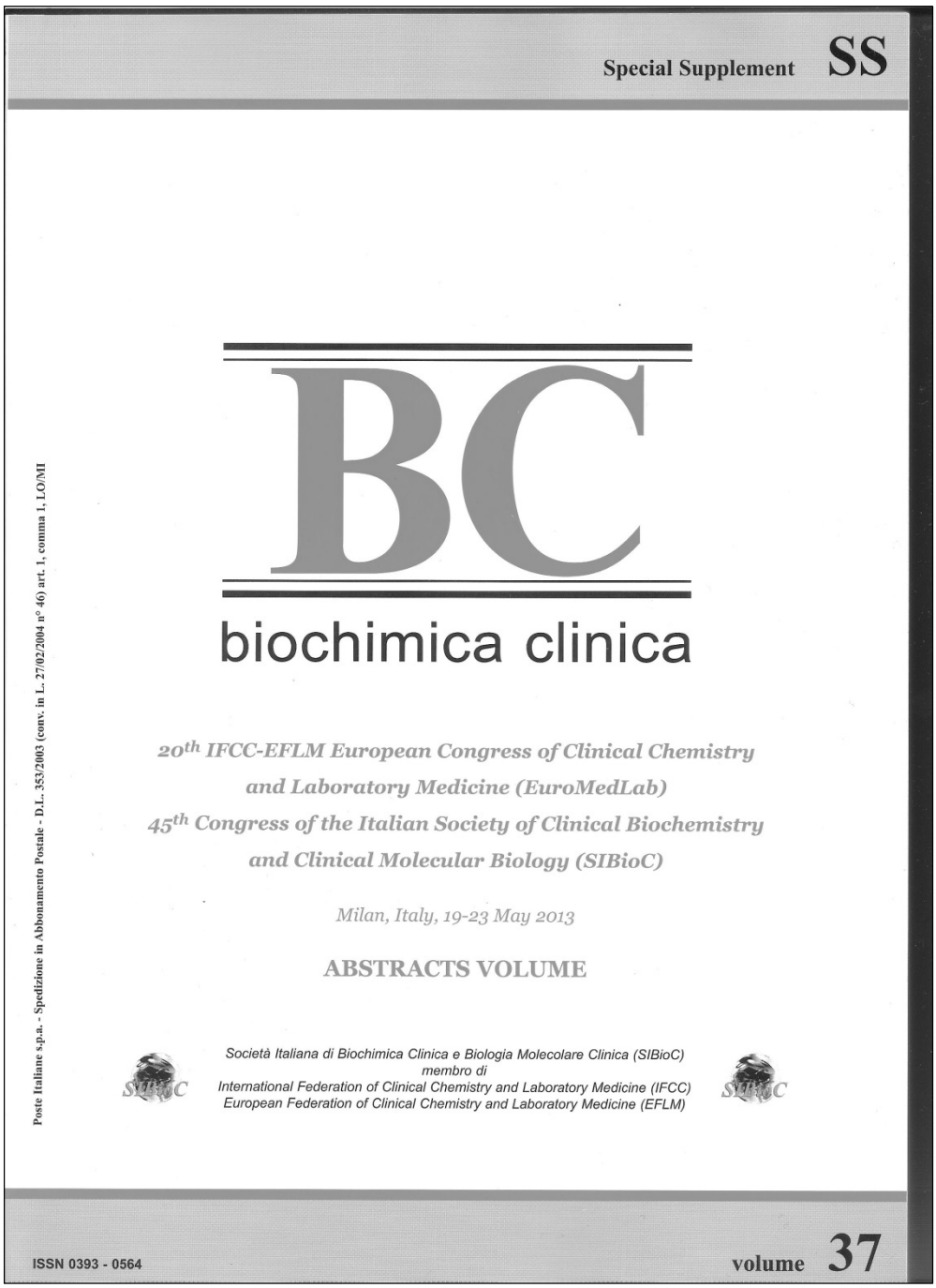
APPENDICES

Appendix A - Publication: IFCCMilan 2013

A.1 Publication: IFCCMilan 2013 – Poster (Journal Cover)



A.2 Publication: IFCCMilan 2013 – Abstract

T270

"MEASUREMENT UNCERTAINTY IN THE MEDICAL LABORATORY - IMPLEMENTATION AND EVALUATION OF TWO DIFFERENT FORMULAS IN CLINICAL CHEMISTRY PARAMETERS: TOTAL CHOLESTEROL, CREATININE AND GLUCOSE MEASUREMENTS."

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[5]*Gnóstica, Clinical Laboratory*

Background: Laboratory Accreditation by ISO:15189 requires the application of uncertainty calculation in medical laboratories. Specifically it requires laboratories to calculate this data, and to provide patient results along with their associated uncertainty values. The Guide to the expression of uncertainty in measurement describes in general terms the calculation of measurement uncertainties (MU); however, the specific aspects of clinical laboratory testing are not addressed. Several models of uncertainty have been studied, but there is no consensus on implementation.
Methods: The aim of the study was to investigate two potential MU formulas (MU-A and MU-B) within a Portuguese ISO:15189 Accredited Clinical Laboratory. Results were compared with Total Error, currently accepted by the Portuguese Institute for Accreditation as MU value. Three accredited analyte measurements were considered: Total Cholesterol, Creatinine and Glucose, measured in human serum samples using two Cobas® 6000–c501(Roche®) analysers.
Results: Cholesterol - Normal: TE=4.7; 5.4%; MU-A=4.2; 4.8%; MU-B=4.6; 5.1%; Pathological: TE=4.2; 4.5%; MU-A=3.7; 4.1%; MU-B=4.1; 4.4%; Creatinine - Normal: TE=12.5; 10.9%; MU-A=11.6; 10.4%; MU-B=9.9; 8.5%; Pathological: TE=10.5; 9.9%; MU-A=10.2; 9.8%; MU-B=8.2; 7.7%; Glucose - Normal: TE=3.9; 4.4%; MU-A=3.6; 4.0%; MU-B=7.0; 7.2%; Pathological: TE=3.9; 4.6%; MU-A=3.6; 4.0%; MU-B=7.0; 7.3%
Conclusions: MU-B formula was capable to provide reliable values, allowing definition of procedure's variability, well representing the dispersion of values reasonably attributable to the measurand final result. Became clear the necessary investment from manufacturers, Reference Laboratories and International Organisations to promote and produce certified reference material with high metrological traceability, focusing values at levels of critical decision, with uncertainties associated to the assigned values. General laboratory investment is also needed to improve practice in the pre-analytical phase, but also to assess and evaluate their own specific pre-analytical uncertainty. In addition, guidelines and tables with new goals/limits, defined according to the evaluation methodologies and tools being introduced in the clinical laboratory, must be developed.

A.3 Publication: IFCCMilan 2013 – Poster

"Measurement Uncertainty in the Medical Laboratory - Implementation and evaluation of two different formulas in Clinical Chemistry parameters: Total Cholesterol, Creatinine and Glucose measurements"

Raposo, R.P.[1]; Freitas, A.M.M.S.[2]; Gomes, M.A.[3]; Morgan, S. L.[3]; Falesa, M.[4]
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INTRODUTION:

The International Vocabulary of Metrology (VIM) [1] defines the concept of uncertainty as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. The GUM: Guide to the expression of uncertainty in measurement [2] describes in general terms the calculation of measurement uncertainties (MU); however, the specific aspects and conditions regarding clinical laboratory testing are excluded, or simply not addressed. Laboratory Accreditation, by ISO 15189:2007 [3], specifies the application of the concept of uncertainties in the medical laboratory, considering the need to have such data and to provide patients their results with the information of the uncertainty values associated to them. The intention is to make medical laboratory measurements and results transferable, or comparable, on a global basis, stating the idea that a good estimate of measurement uncertainty is necessary: to ensure that the results are traceable to international or national standards; to compare results between laboratories and/or specifications, legal tolerances or regulatory limits; to make informed decisions and improve test methods and those same results, assuring that they are fit for purpose. Thus, several models have been studied over last years and its application has been subjected to several contradictory arguments, not yet resulting in a consensus on its implementation. Although encouraged, the determination of measurement uncertainty is not a common practice in the laboratory tests in health, raising yet too many questions, and having its application been subjected to several contradictory arguments since the publication of the GUM.

METHODOLOGY:

This experimental design has adapted and implemented two different MU formulas (MU-A and MU-B), of the Portuguese Clinical Laboratory, Accredited by the NP EN ISO 15189:2007 [4]. Along with the application of the two different models, were also considered for comparison the values for Total Error (TE), which are currently accepted by the Portuguese Institute for Accreditation (IPAC) as uncertainty values in the accreditation process, with the objective of comparing what is being made with the new approaches, while simultaneously comparing the two different budget findings and results. We have considered, for this purpose, three Accredited analyte measurements, being Total Cholesterol, Creatinine and Glucose, for both pathological and normal control values, all of them measured in human serum, in two Cobas® 6000 – c501 module (Roche®), operating in the same laboratory under the same conditions, handled by the same technicians and with the same reagents, controls and calibration material (Cobas® c501/c502/c311/c701/c702 - C.a.s.f.), also from Roche®.

In each one of the formulas different sources or categories of uncertainty contributed with a partial value, always according to a *Top-Down* approach, considered to be the most suitable or appropriate for the clinical laboratory. This kind of approach generally is recognised as a direct estimate of the combined standard uncertainty of the whole procedure, respecting the principles of the GUM, but where the calculation is not based on the identification and knowledge of the all the different individual sources of uncertainty or in the use of different mathematical models for the determinations of each standard uncertainty.

The sources of uncertainty within a medical laboratory result are many and varied but, focusing on a top-down approach, can all be embedded under the broad categories of Pre-Analytical Variation, Analytical Variation and Biological Variation, considering that all sources of uncertainty are being accounted, somewhere along the way, within the appropriate category.

RESULTS AND DISCUSSION:

The results from the Quality System were considered, particularly the data from the Internal Quality Control (IQC) and from the External Quality Assessment (EQA) Program (RIQAS®). From here, it was possible to calculate the Total Error (TE). These results are compared against the reference values for allowable total error (TEa), from international tables and guidelines, such as the "Desirable Biological Variation Database Specifications"[5] or the "CLIA regulations table"[6].

Test	Medical Decision Level a)	IQC (X mg/dL) -Normal-	IQC (X mg/dL) -Pathological-	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-	Inaccuracy (|BIAS|%)	Total Error -Normal- (n=2) %	Total Error -Pathological- (n=2) %	European Table (TEa) %	CLIA (TEa) %
CH (C1)	190 mg/dl	95,76	180,06	1,88	1,62	0,95	4,71	4,18	8,5	10
CH (C2)	190 mg/dl	95,94	180,45	2,20	1,80	0,95	5,35	4,55	8,5	10
CR (C1)	1,2 mg/dl	1,10	3,78	4,31	3,31	3,85	12,47	10,47	8,9	15
CR (C2)	1,2 mg/dl	1,12	3,82	3,51	3,03	3,85	10,87	9,92	8,9	15
GL (C1)	110 mg/dl	97,44	233,60	1,36	1,36	2,15	3,87	3,87	6,9	10
GL (C2)	110 mg/dl	97,98	234,72	1,63	1,75	2,15	4,40	4,65	6,9	10

C1 - Cobas® 6000 - c501 Module Equipment 1 C2 - Cobas® 6000 - c501 Module Equipment 2

a) Medical Decision Level: quality system / laboratory's reference values.

Table 1 - Quality System Data and Total Error Determination

Test / Analyte	Imprecision (CV%) -Normal-	Imprecision (CV%) -Pathological-	Inaccuracy (|BIAS|%)	Calibrator Expanded Uncertainty (mg/dL)	Calibrator Value (mg/dL)	Calibrator Standard Uncertainty (%)	Pre-analytical Uncertainty (%)
Cholesterol-T (C1)	1,88	1,62	0,95	1,46	165,00	0,44	1,20
Cholesterol-T (C2)	2,20	1,80	0,95	1,46	165,00	0,44	1,20
Creatine (C1)	4,31	3,31	3,85	0,0576	4,06	0,71	2,30
Creatine (C2)	3,51	3,03	3,85	0,0576	4,06	0,71	2,30
Glucose (C1)	1,36	1,36	1,15	1,63	193,00	0,42	3,20
Glucose (C2)	1,63	1,75	1,15	1,63	193,00	0,42	3,20

C1 - Cobas 6000® - c501 Module Equipment 1 / C2 - Cobas® 6000 - c501 Module Equipment 2

Table 2 - Imprecision, Bias, Calibrator Uncertainty and Pre-analytical Uncertainty

Combining these data, we could apply directly the Model-A formula. After gathering information on the values of uncertainty assigned to the calibrator, from the manufacturers certificate (Roche®), and of the pre-metrological variability for these same analytes, from the literature [7, 8], it was possible to apply the formula for Model-B.

Test / Analyte	Total Error -Normal- (n=2)	Total Error -Pathological- (n=2)	MU (A) -Normal-	MU (A) -Pathological-	MU (B) -Normal-	MU (B) -Pathological-	European Table (TEa) %	CLIA (TEa) %
Cholesterol-T (C1)	4,71	4,18	4,2	3,7	4,6	4,1	8,5	10
Cholesterol-T (C2)	5,35	4,55	4,8	4,1	5,1	4,4	8,5	10
Creatine (C1)	12,47	10,47	11,6	10,2	9,9	8,2	8,9	15
Creatine (C2)	10,87	9,92	10,4	9,8	8,5	7,7	8,9	15
Glucose (C1)	3,87	3,87	3,6	3,6	7,0	7,0	6,9	10
Glucose (C2)	4,40	4,65	4,0	4,2	7,2	7,3	6,9	10

C1 - Cobas 6000® - c501 Module Equipment 1 / C2 - Cobas® 6000 - c501 Module Equipment 2

Table 3 - Total Error, MU Model-A and MU Model-B

The Model-A formula for MU does not introduce any new concepts to the common evaluation on TE, using the exact same data, it just applies the rules of uncertainty propagation and combination. Concerning the Model-B formula, it includes something new, adding the variability/uncertainty associated with the pre-metrological procedures, as well as the uncertainty assigned to the calibration material. Simultaneously, it disregards the Bias value.

CONCLUSION:

- Although we can easily associate the results and formula for the Model-A with the determination of TE and, even if with some restrictions, use the same tables and guidelines to evaluate the methods performance, it is evident that there is information missing and uncertainty sources being neglected, or not considered.
- We found the MU-B formula capable to produce sustainable outcomes, providing reliable and realistic values of measurement uncertainty, allowing the defining of the procedure variability and well representing the dispersion of values that can reasonably be attributed to the final measurand result.
- Nevertheless, became clear the necessary investment from manufacturers, reference laboratories and international organizations to promote and produce certified reference material with high metrological traceability, focusing on values at levels of critical decision, providing these materials with the uncertainties associated to their assigned values. Also for general laboratories to invest as well, mostly to improve their practice in the pre-analytical phase but also to assess and evaluate their own pre-analytical or pre-metrological uncertainty.
- It is vital that international scientific organizations and governments invest in studying and developing new guidelines and tables with new goals and limits, defined according to the new evaluation methodologies and tools being introduced in the medical laboratory. It is obvious the necessity of actualization of the ones in use, with the incorporation of the concept and determination of uncertainty and introduction of new requisites for performance and maximum allowable values.
- The combination of all the above would produce an undoubted improvement to the general and total quality and reliability of the medical laboratories procedures, performances and consequently of its results, enhancing traceability and comparability of methods, laboratories and results, and in the end lead to a better and stronger laboratory services and health care.

REFERENCES:

Appendix B - Publication: IFCC Berlin2011

B.1 Publication: IFCCBerlin – Abstract

Poster Abstracts – IFCC – WorldLab – EuroMedLab Berlin 2011 – Berlin, 15-19 May 2011 • DOI 10.1515/CCLM.2011.527 • S732
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Quality assessment, standardization

1054

QUALITY INDICATORS IN A CLINICAL LABORATORY: FROM CERTIFICATION TO ACCREDITATION

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Background. Quality indicators are important management tools that can be used to evaluate the organization's overall performance and effectiveness, enabling to examine/follow the evolution of the quality of its services.
Methods. To evaluate the results of the Quality Management System in the laboratory, throughout the upgrade from Certification (ISO 9001:2000) to Accreditation (NP EN ISO15189:2007), main quality indicators from Pre-Analytical, Analytical, and Post-Analytical phases was analyzed, from 2002 to 2009.
Results. The waiting time was reduced from 21.2 minutes (2005) to 11.9 minutes (2009); the repetitions of sample collecting decreased from 1.4% (2002) to 0.91% (2009). Repeat testing decreased from 14% (2002) to 1.98% (2009), being responsible for a reduction of 17% in the cost of reagents per analysis. The correct results in the External Quality Assessment Schemes were 90.45% in 2003 and 96.3% in 2009. The imprecision (CV%) decreased since 2003 to 2009, with an achievement of target values of 72.8% and 79.7%, respectively. In 2009, 77% of Total Error determination had an evaluation between Good and Excellent. The errors detected after the emission of the results decreased from 0.15% (2003) to 0.064% (2009). During this period the laboratory users increased over 60%, from 36 640 Users (2003) to 59,005 (2009) and an increase in the number of analysis / tests from 330 441 to 596 655.
Conclusions. The study provided evidence that the implementation ISO Standards for Quality Systems in Clinical Laboratories (ISO9001 and ISO15189) it's very important to monitor the laboratory's overall contribution to patient care.

B.2 Publication: IFCCBerlin – Poster

Universidade do Algarve
Escola Superior de Saúde

QUALITY INDICATORS IN A CLINICAL LABORATORY: FROM CERTIFICATION TO ACCREDITATION

Raposo, R.P.[1]; Freitas, A.M.M.S.[1]; Falaca, M. [2]; Gomes, A.[3] and Cavaco, I.[4]
[1] Escola Superior de Saúde da Universidade do Algarve, Faro, Portugal; [2] Gnóstica – Laboratório de Análises Clínicas, Faro, Portugal; [3] INSA, Lisboa, Portugal; [4] FCT, Universidade do Algarve, Faro, Portugal.

INTRODUTION:

Quality indicators are important management tools that can be used to evaluate the organization's overall performance and effectiveness. The observation in detail of specific aspects of performance enables to examine/follow the evolution of the quality of the services, allowing to improve and reach higher standards in way to Total Quality. In a private clinical laboratory a retrospective study was performed, based on the Quality Management System data, to evaluate the several quality indicators available. During this period, we studied the evolution of the Quality Management System as the Laboratory went through two processes: Certification by the standard: NP EN ISO 9001:2000 [P1], and later, Accreditation by the standard NP EN ISO15189:2006 [P2].

METHODS

From 2002 to 2009, throughout the process of certification to accreditation (Figure 1), the study included the different phases of the laboratory process and the use of tools to evaluate each step of the Pre-Analytical, Analytical and Post-Analytical phases. Gathering all of this information made it possible to isolate and study a lot of different variables (Figure 2).

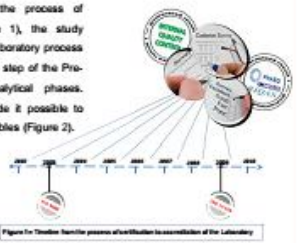


Figure 1 – Timeline from the process of certification to accreditation of the Laboratory

The performance of the Laboratory, as well as the user's satisfaction and quality perception were assessed by the results of an anonymous questionnaire, applied every year. Being a part of the Laboratory policy of quality, a total of 2237 completed surveys were analysed, during this period.

The information on the Management System was considered, to analyse the evolution of the Laboratory regarding the number of users/clients and numbers of tests/analysis through the years. Critical errors in the laboratory process and the resulting consequences, in terms reagents/materials consumption and costs were also studied.

Referring to the pre-analytical phase the waiting time of users, the errors made on inscriptions (in user's data/identification or tests requisitions, such as wrong names or the change/absence of analyses from the request) were analysed. On the analytical phase, to follow the evolution of the quality indicators were controlled the numbers of repeat testing and evaluated the results from the Internal Quality Control and from the External Quality Assessment Schemes. Finally, on the post-analytical phase, we studied the records of the execution time of the analysis and the delivery of results, as well as the perception of users upon the presentation of them.

Figure 2 – Quality indicators analysed in the study

RESULTS AND DISCUSSION:

The assembled information, besides plenty of suggestions directly from the users, brought a lot of improvement opportunities, becoming parting points to some of the progress made in the pursuit for a better service with higher quality standards:

- The number of repetitions of sample collecting decrease from 1.4% (2002) to 0.91% (2009), and the waiting time in the Laboratory was reduced to 11.9 minutes, after having reached a maximum record in 2006, with 25 minutes (Figure 3);

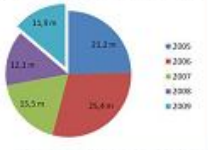


Figure 3 – Evolution of user's waiting time in the Laboratory

- There was a great improvement in repeat testing, dropping from 14% (2002), to 1.98% (2009), with a consequent and relevant decrease of values, from over 30% to 13%, in reagents cost per analysis regarding the billing, in the same period (Figure 4);

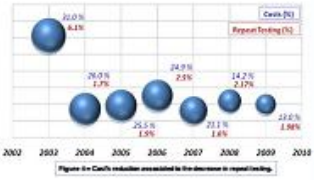


Figure 4 – Costs reduction associated to the decrease in repeat testing.

- The imprecision (CV%), raised from 64% (2006) to 91.6% (2009) of the results meeting the desirable specification values [P3][P4]. Bias determination revealed a slight improvement, from 89.9% to 90.4%, in the same period and according to the same tables (Figure 5);
- On External Quality Assessment Programs, the correct results of the participation in 2003 (22 different schemes) and in 2009 (24 programs completed) improved from 90.45% to 96.3%;
- The Total Error, in 2009 had 80.8% of results with the evaluation of Good or Excellent, in opposite to the 58,1% obtained in 2006 [P4] (Figure 6);

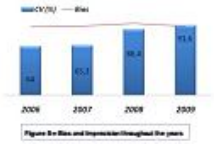


Figure 5 – Bias and Imprecision throughout the years

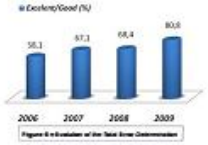


Figure 6 – Evolution of the Total Error Determination

- In an overall analysis, it's important to highlight the record of 0.064% (2009) non-conformities, in comparison with 0.15% (2003). The majority of non-conformities (78,9%) have origin in the reception, revealing a weak point and an opportunity to improve (Figure 7).
- Throughout the certification to accreditation there was a growth of the number of inscriptions in the laboratory, resulting in an expansion over 60%, from 36640 users (2003) to 59005 (2009) and an increase in the number of analysis / tests from 330441 to 596655, during the same period (Figure 8).

Figure 7 – Decrease of the non-conformities (%)

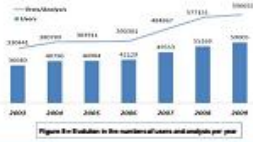


Figure 8 – Evolution in the numbers of users and analysis per year

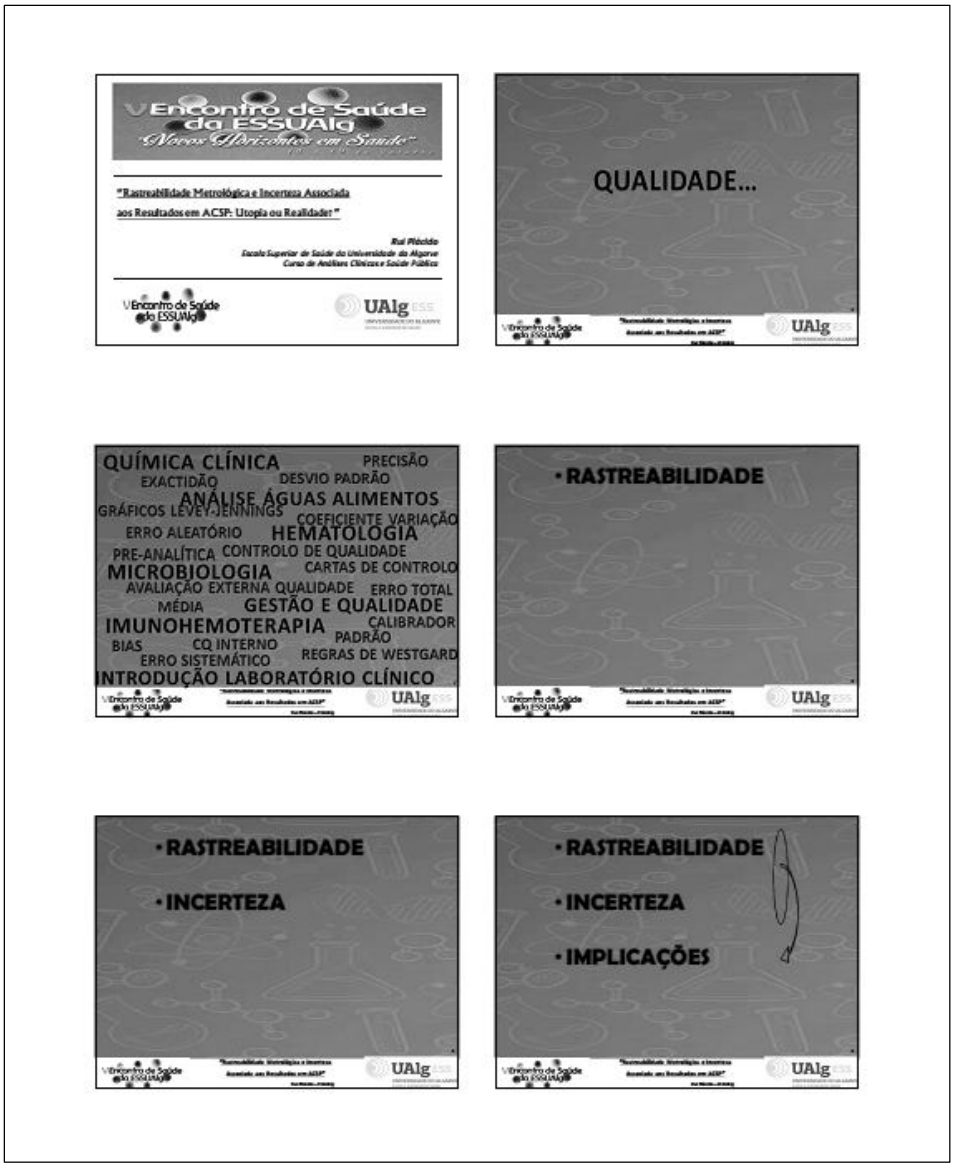
CONCLUSION:

- The quality indicators revealed the real improvements brought by the Certification, reinforced later by the Accreditation process. There is still much work ahead, but this seems to be the way in the search for Total Quality, by listening to the users and monitoring the quality indicators, to follow up and meet the ISO guidelines.
- The implementation of user-focused policies, responding to their criticisms and suggestions, allowed the application of corrective measures, namely in the reduction of waiting time, transforming the weaknesses in improvements and strong points to the whole system, leading to a minimum registries of non-conformities .
- As a consequence, there was an increase in the number of users and analysis per year and a reduction of costs due to the decrease in the number of test and sample collection repeating.
- This work policy also led to concrete and measurable results in the analytical quality control, such as the observed improvements in imprecision, bias and total error determination.
- The present study brings evidence that the implementation of ISO Standards for Quality Systems in Clinical Laboratories (ISO9001 and ISO15189) is very important to monitor the laboratory's overall contribution to patient care, providing tools to improve the quality of services, processes and results, leading ultimately to relevant reductions in non-quality costs.

REFERENCES:

Appendix C - Meeting: "New Horizons in Health" School of Health – University of Algarve - IV Health (Presentation)

C.1 Presentation: "*Metrological Traceability and uncertainty associated with the results in the Medical Laboratory: Reality or Utopia ?!"*



- RASTREABILIDADE
- INCERTEZA
- IMPLICAÇÕES

PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES

- RASTREABILIDADE
- INCERTEZA
- IMPLICAÇÕES

PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES

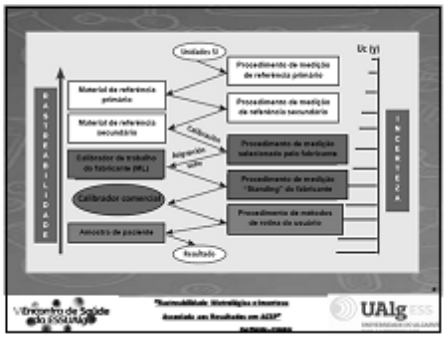
VIM:

VOCABULÁRIO INTERNACIONAL METROLOGIA

VIM:

(2.2)
METROLOGIA – Ciência da medição e suas aplicações.

(2.41)
RASTREABILIDADE METROLÓGICA
- Propriedade de um resultado de medição através da qual o resultado pode ser relacionado a uma referência por intermédio de uma cadeia ininterrupta e documentada de calibrações, cada uma contribuindo para a incerteza de medição .

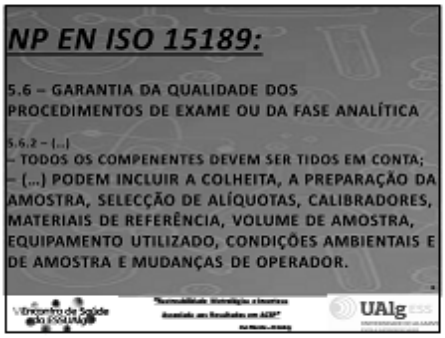

Unidades SI
Uc (y)
Procedimento de medição de referência primário
Material de referência primário
Procedimento de medição de referência secundário
Material de referência secundário
Calibração
Calibrador de trabalho do fabricante (MC)
Procedimento de medição calibrado pelo fabricante
Interpolação
Procedimento de medição "Standing" do fabricante
Calibrador comercial
Procedimento de métodos de rotina do usuário
Amostra de paciente
Resultado
RASTREABILIDADE
INCERTEZA


UNIÃO EUROPEIA:
DIRECTIVA IVD-MD 98/79/EC
- (…) OBRIGAÇÃO DOS FABRICANTES EM GARANTIR A RASTREABILIDADE DOS SEUS SISTEMAS ANALÍTICOS PARA REFERÊNCIAS DE ORDEM RECONHECIDAMENTE SUPERIOR, INDICANDO INCERTEZA ASSOCIADA AOS CALIBRADORES/MATERIAIS DE REFERÊNCIA.


DIRECTIVA IVD-MD 98/79/EC
NORMAS ISO RELACIONADAS:
- ISO 17511 – METROLOGICAL TRACEABILITY OS VALUES ASSIGNED TO CALIBRATORS AND CONTROLS
- ISO 15193 – REFERENCE MEASUREMENT PROCEDURES
- ISO 15194 – DESCRIPTION OF REFERENCE MATERIALS
- ISO 15195 – REQUIREMENTS FOR REFERENCE MEASUREMENT LABORATORIES
- ISO 18153 – METROLOGICAL TRACEABILITY OF VALUES FOR CATALYTIC CONCENTRATION OF ENZIMES (…)
- (…)

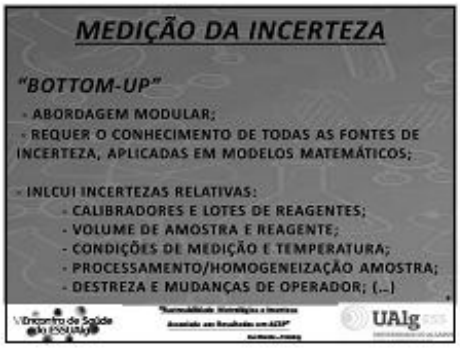

NP EN ISO 15189:
LABORATÓRIOS CLÍNICOS – REQUISITOS PARTICULARES DE QUALIDADE E COMPETÊNCIA.
Norma Portuguesa


NP EN ISO 15189:
LABORATÓRIOS CLÍNICOS
- REQUISITOS PARTICULARES DE QUALIDADE E COMPETÊNCIA.
5.6 – GARANTIA DA QUALIDADE DOS PROCEDIMENTOS DE EXAME OU DA FASE ANALÍTICA
5.6.2 – (…) DETERMINAÇÃO DA INCERTEZA…


NP EN ISO 15189:
5.6 – GARANTIA DA QUALIDADE DOS PROCEDIMENTOS DE EXAME OU DA FASE ANALÍTICA
5.6.2 – (…)
– TODOS OS COMPENENTES DEVEM SER TIDOS EM CONTA;
– (…) PODEM INCLUIR A COLHEITA, A PREPARAÇÃO DA AMOSTRA, SELECÇÃO DE ALÍQUOTAS, CALIBRADORES, MATERIAIS DE REFERÊNCIA, VOLUME DE AMOSTRA, EQUIPAMENTO UTILIZADO, CONDIÇÕES AMBIENTAIS E DE AMOSTRA E MUDANÇAS DE OPERADOR.


•RASTREABILIDADE
•INCERTEZA
•IMPLICAÇÕES
PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES
UAlg

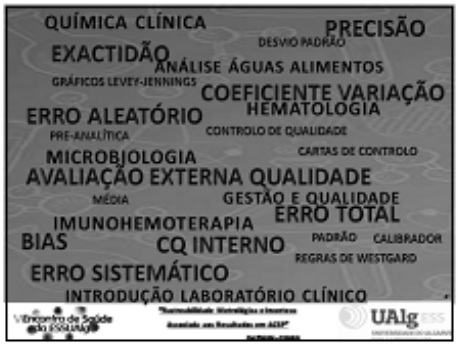

GUM GUIDE UNCERTAINTY MEASUREMENT
INCERTEZA:
"um parâmetro não negativo que caracteriza a dispersão dos valores atribuídos a um mensurando, com base nas informações utilizadas".
UAlg


MEDIÇÃO DA INCERTEZA
"BOTTOM-UP"
- ABORDAGEM MODULAR;
- REQUER O CONHECIMENTO DE TODAS AS FONTES DE INCERTEZA, APLICADAS EM MODELOS MATEMÁTICOS;
- INLCUI INCERTEZAS RELATIVAS:
- CALIBRADORES E LOTES DE REAGENTES;
- VOLUME DE AMOSTRA E REAGENTE;
- CONDIÇÕES DE MEDIÇÃO E TEMPERATURA;
- PROCESSAMENTO/HOMOGENEIZAÇÃO AMOSTRA;
- DESTREZA E MUDANÇAS DE OPERADOR; (...)
UAlg


MEDIÇÃO DA INCERTEZA
"TOP-DOWN"
- ABORDAGEM EMPÍRICA;
- AGRUPA AS INCERTEZAS EM DIFERENTES CATEGORIAS;
- CONSIDERA A PERFORMANCE COMPLETA: MÉTODO/OPERADOR;
- RECORRE A LINGUAGEM E DADOS DO DIA-A-DIA LABORATORIAL;
- CONSIDERADA MAIS ADEQUADA E APLICÁVEL.
UAlg


MEDIÇÃO DA INCERTEZA
"TOP-DOWN"
- RECORRE A LINGUAGEM E DADOS DO DIA-A-DIA LABORATORIAL;
- CONSIDERADA MAIS ADEQUADA E APLICÁVEL.
UAlg


QUÍMICA CLÍNICA
PRECISÃO
EXACTIDÃO
DESVIO PADRÃO
ANÁLISE ÁGUAS ALIMENTOS
GRÁFICOS LEVEY-JENNINGS
COEFICIENTE VARIAÇÃO
ERRO ALEATÓRIO
HEMATOLOGIA
PRE-ANALÍTICA
CONTROLO DE QUALIDADE
MICROBIOLOGIA
CARTAS DE CONTROLO
AVALIAÇÃO EXTERNA QUALIDADE
ERRO TOTAL
MÉDIA
GESTÃO E QUALIDADE
IMUNOHEMOTERAPIA
CALIBRADOR
PADRÃO
BIAS
CQ INTERNO
ERRO SISTEMÁTICO
REGRAS DE WESTGARD
INTRODUÇÃO LABORATÓRIO CLÍNICO
UAlg


QUÍMICA CLÍNICA
PRECISÃO
DESVIO PADRÃO
EXACTIDÃO
ANÁLISE ÁGUAS ALIMENTOS
GRÁFICOS LEVEY-JENNINGS
COEFICIENTE VARIAÇÃO
ERRO ALEATÓRIO
HEMATOLOGIA
PRE-ANALÍTICA
CONTROLO DE QUALIDADE
MICROBIOLOGIA
CARTAS DE CONTROLO
AVALIAÇÃO EXTERNA QUALIDADE
MÉDIA
GESTÃO E QUALIDADE
ERRO TOTAL
IMUNOHEMOTERAPIA
BIAS
CQ INTERNO
PADRÃO
CALIBRADOR
REGRAS DE WESTGARD
ERRO SISTEMÁTICO
INTRODUÇÃO LABORATÓRIO CLÍNICO


PRECISÃO
EXACTIDÃO
ERRO ALEATÓRIO
ERRO SISTEMÁTICO
CQ INTERNO
AVALIAÇÃO EXTERNA QUALIDADE
COEFICIENTE VARIAÇÃO
BIAS
ERRO TOTAL

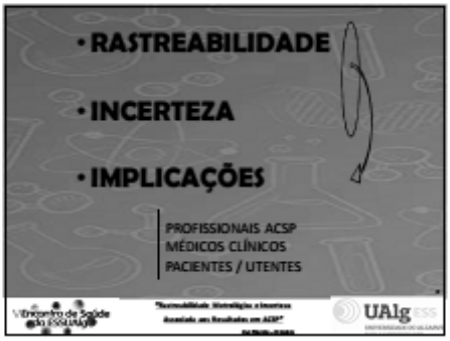

TABELAS REGULAÇÃO/COMPARAÇÃO
CLIA Proficiency Testing criteria
CLIA FINAL RULES FOR QUALITY SYSTEMS
Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation


QUÍMICA CLÍNICA
PRECISÃO
DESVIO PADRÃO
EXACTIDÃO
ANÁLISE ÁGUAS ALIMENTOS
GRÁFICOS LEVEY-JENNINGS
COEFICIENTE VARIAÇÃO
ERRO ALEATÓRIO
HEMATOLOGIA
CONTROLO DE QUALIDADE
PRE-ANALÍTICA
MICROBIOLOGIA
CARTAS DE CONTROLO
AVALIAÇÃO EXTERNA QUALIDADE
MÉDIA
GESTÃO E QUALIDADE
IMUNOHEMOTERAPIA
ERRO TOTAL
BIAS
CQ INTERNO
PADRÃO
CALIBRADOR
ERRO SISTEMÁTICO
REGRAS DE WESTGARD
INTRODUÇÃO LABORATÓRIO CLÍNICO


EXACTIDÃO
PRECISÃO
COEFICIENTE VARIAÇÃO
ERRO ALEATÓRIO
PRE-ANALÍTICA
BIAS
INCERTEZA
CQ INTERNO
PADRÃO
CALIBRADOR
ERRO SISTEMÁTICO
AVALIAÇÃO EXTERNA QUALIDADE


IFCC WORLDLAB BERLIN2011:
Measurement uncertainty components
Pre-metrological variability
Measurement uncertainty of the calibrator
Assigned quantity value
Day-to-day imprecision

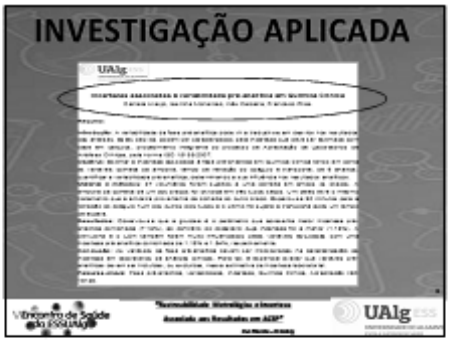

EUROMEDLAB
MILANO 2013
19-23 MAY
How to estimate the measurement uncertainty in clinical laboratories: is the GUM approach mandatory?
Ferruccio Ceriotti
Diagnostica e Ricerca San Raffaele, Milano
SIBioC Working Group on Analytical Quality
FERRUCCIO CERIOTTI – MEMBRO IFCC / EFCC; VICE PRESIDENTE SIBioC.
UAlg


• RASTREABILIDADE
• INCERTEZA
• IMPLICAÇÕES
PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES
UAlg

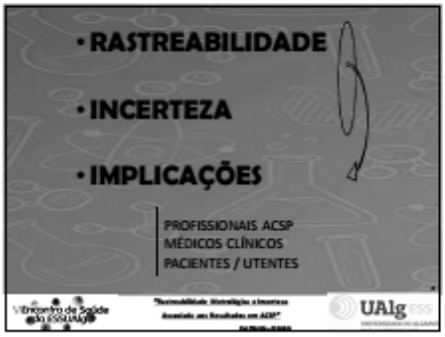

INCERTEZA DE MEDIÇÃO
FÓRMULA/MÉTODO:
- PODE/DEVE SER "AFINADA";
- OUTROS FACTORES / FONTES DE INCERTEZA CONSIDERADOS;
- VARIAÇÃO BIOLÓGICA – COMPARAÇÃO DE TESTES DO MESMO PACIENTE OU MONITORIZAÇÃO DE DOENÇAS CRÓNICAS.
UAlg


INCERTEZA DE MEDIÇÃO
FÓRMULA/MÉTODO:
- PODE/DEVE SER "AFINADA";
(...)
- LABORATÓRIOS:
PAPEL FUNDAMENTAL
UAlg

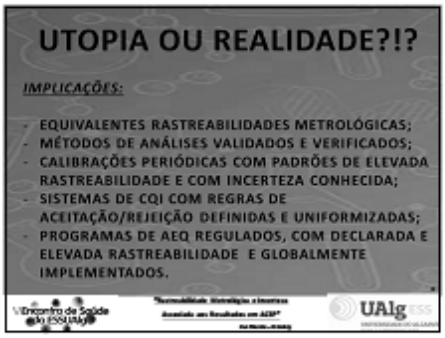

INCERTEZA DE MEDIÇÃO
- LABORATÓRIOS:
PAPEL FUNDAMENTAL
- DIRECTIVA IVD-MD 98/79/EC
- ISO 17511 – METROLOGICAL TRACEABILITY OS VALUES ASSIGNED TO CALIBRATORS AND CONTROLS
- ISO 15193 – REFERENCE MEASUREMENT PROCEDURES
- ISO 15194 – DESCRIPTION OF REFERENCE MATERIALS
- ISO 15195 – REQUIREMENTS FOR REFERENCE MEASUREMENT LABORATORIES
- ISO 18153 – METROLOGICAL TRACEABILITY OF VALUES FOR CATALYTIC CONCENTRATION OF ENZIMES (...)
UAlg

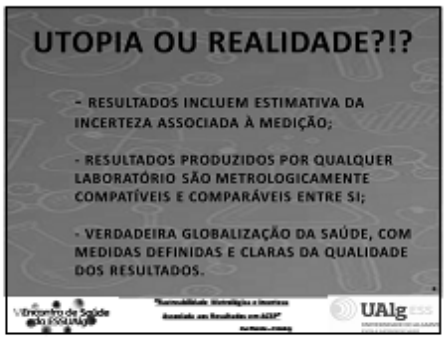

INVESTIGAÇÃO APLICADA
UAlg

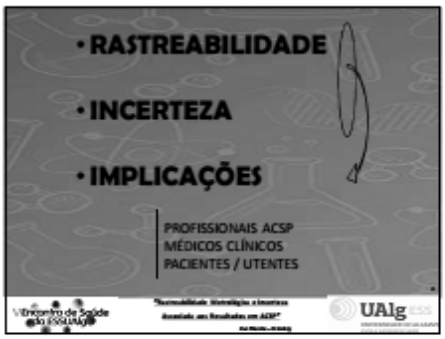

INCERTEZA DE MEDIÇÃO
DESAFIO:
- SEGUIR O CAMINHO DA RASTREABILIDADE;
- PERMITIR A COMPARABILIDADE GLOBAL;
- MODELO ACEITE PELOS PARES, COMO APLICÁVEL E ADEQUADO A:
PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES

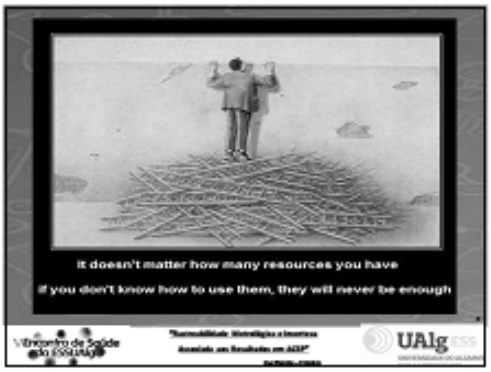

• RASTREABILIDADE
• INCERTEZA
• IMPLICAÇÕES
PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES


UTOPIA OU REALIDADE?!?


UTOPIA OU REALIDADE?!?
IMPLICAÇÕES:
- EQUIVALENTES RASTREABILIDADES METROLÓGICAS;
- MÉTODOS DE ANÁLISES VALIDADOS E VERIFICADOS;
- CALIBRAÇÕES PERIÓDICAS COM PADRÕES DE ELEVADA RASTREABILIDADE E COM INCERTEZA CONHECIDA;
- SISTEMAS DE CQI COM REGRAS DE ACEITAÇÃO/REJEIÇÃO DEFINIDAS E UNIFORMIZADAS;
- PROGRAMAS DE AEQ REGULADOS, COM DECLARADA E ELEVADA RASTREABILIDADE E GLOBALMENTE IMPLEMENTADOS.


UTOPIA OU REALIDADE?!?
- RESULTADOS INCLUEM ESTIMATIVA DA INCERTEZA ASSOCIADA À MEDIÇÃO;
- RESULTADOS PRODUZIDOS POR QUALQUER LABORATÓRIO SÃO METROLOGICAMENTE COMPATÍVEIS E COMPARÁVEIS ENTRE SI;
- VERDADEIRA GLOBALIZAÇÃO DA SAÚDE, COM MEDIDAS DEFINIDAS E CLARAS DA QUALIDADE DOS RESULTADOS.


• RASTREABILIDADE
• INCERTEZA
• IMPLICAÇÕES
PROFISSIONAIS ACSP
MÉDICOS CLÍNICOS
PACIENTES / UTENTES

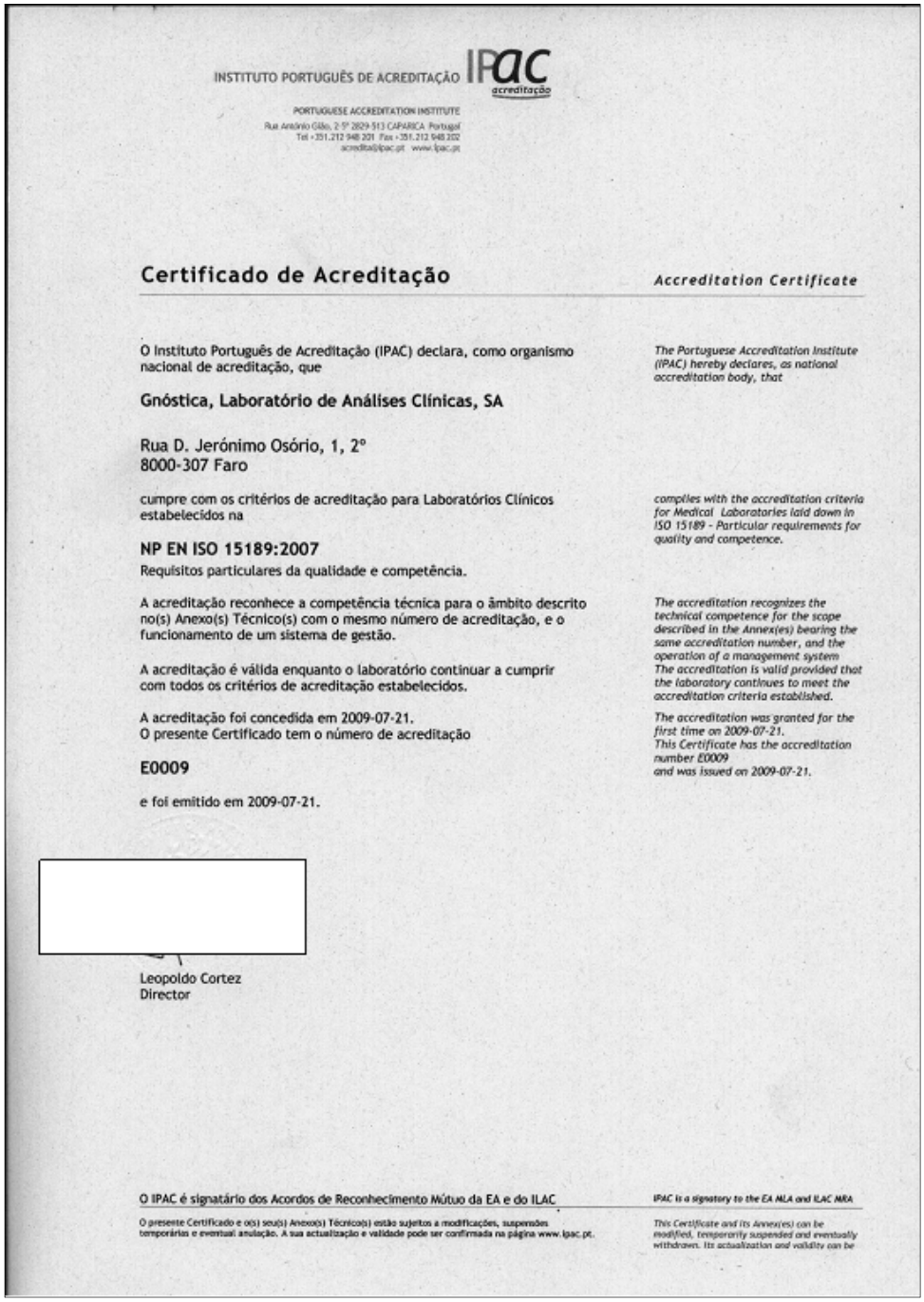


SIGLAS / INSTITUIÇÕES

ARML: Accredited reference measurement laboratory
BIPM Bureau International des Poids et Mesures
BIPM / JCTLM Joint Committee for Traceability in Laboratory Medicine
GUM Guia para a Expressão da Incerteza de Medição
IFCC Federação Internacional de Química Clínica e Biologia Médica
ILAC Cooperação Internacional em Acreditação de Laboratórios
ISO Organização Internacional de Normalização
ISO/REMCO Organização Internacional de Normalização / Comité para os Materiais de Referência
JCGM Comité Comum para os Guias em Metrologia
JCGM/WG 1 Grupo de Trabalho 1 – GUM do Comité Conjunto para os Guias em Metrologia
JCGM/WG 2 Grupo de Trabalho 2 – VIM do Comité Conjunto para os Guias em Metrologia
MI Manufacturer's laboratory
NIST National Institute of Standards and Technology
NMI National metrology institute
OIML Organização Internacional de Metrologia Legal
VIM, 3ª edição Vocabulário Internacional de Metrologia - Conceitos Básicos e Gerais e Termos Associados (2007)

Appendix D - Accreditation Certificate (Year2009)

D.1 Gnóstica Medical Laboratory - Accreditation Certificate: ISO 15189:2007 (IPAC - 2009/07/01)



INSTITUTO PORTUGUÊS DE ACREDITAÇÃO IPAC acreditação

PORTUGUESE ACCREDITATION INSTITUTE
Rua António Gião, 2 5º 2829-513 CAPARICA Portugal
Tel +351.212 948 201 Fax +351.212 948 202
acreditat@ipac.pt www.ipac.pt

Certificado de Acreditação — *Accreditation Certificate*

O Instituto Português de Acreditação (IPAC) declara, como organismo nacional de acreditação, que

The Portuguese Accreditation Institute (IPAC) hereby declares, as national accreditation body, that

Gnóstica, Laboratório de Análises Clínicas, SA

Rua D. Jerónimo Osório, 1, 2º
8000-307 Faro

cumpre com os critérios de acreditação para Laboratórios Clínicos estabelecidos na

complies with the accreditation criteria for Medical Laboratories laid down in ISO 15189 - Particular requirements for quality and competence.

NP EN ISO 15189:2007
Requisitos particulares da qualidade e competência.

A acreditação reconhece a competência técnica para o âmbito descrito no(s) Anexo(s) Técnico(s) com o mesmo número de acreditação, e o funcionamento de um sistema de gestão.

The accreditation recognizes the technical competence for the scope described in the Annex(es) bearing the same accreditation number, and the operation of a management system

A acreditação é válida enquanto o laboratório continuar a cumprir com todos os critérios de acreditação estabelecidos.

The accreditation is valid provided that the laboratory continues to meet the accreditation criteria established.

A acreditação foi concedida em 2009-07-21.
O presente Certificado tem o número de acreditação

The accreditation was granted for the first time on 2009-07-21.
This Certificate has the accreditation number E0009

E0009

e foi emitido em 2009-07-21.

and was issued on 2009-07-21.

Leopoldo Cortez
Director

O IPAC é signatário dos Acordos de Reconhecimento Mútuo da EA e do ILAC

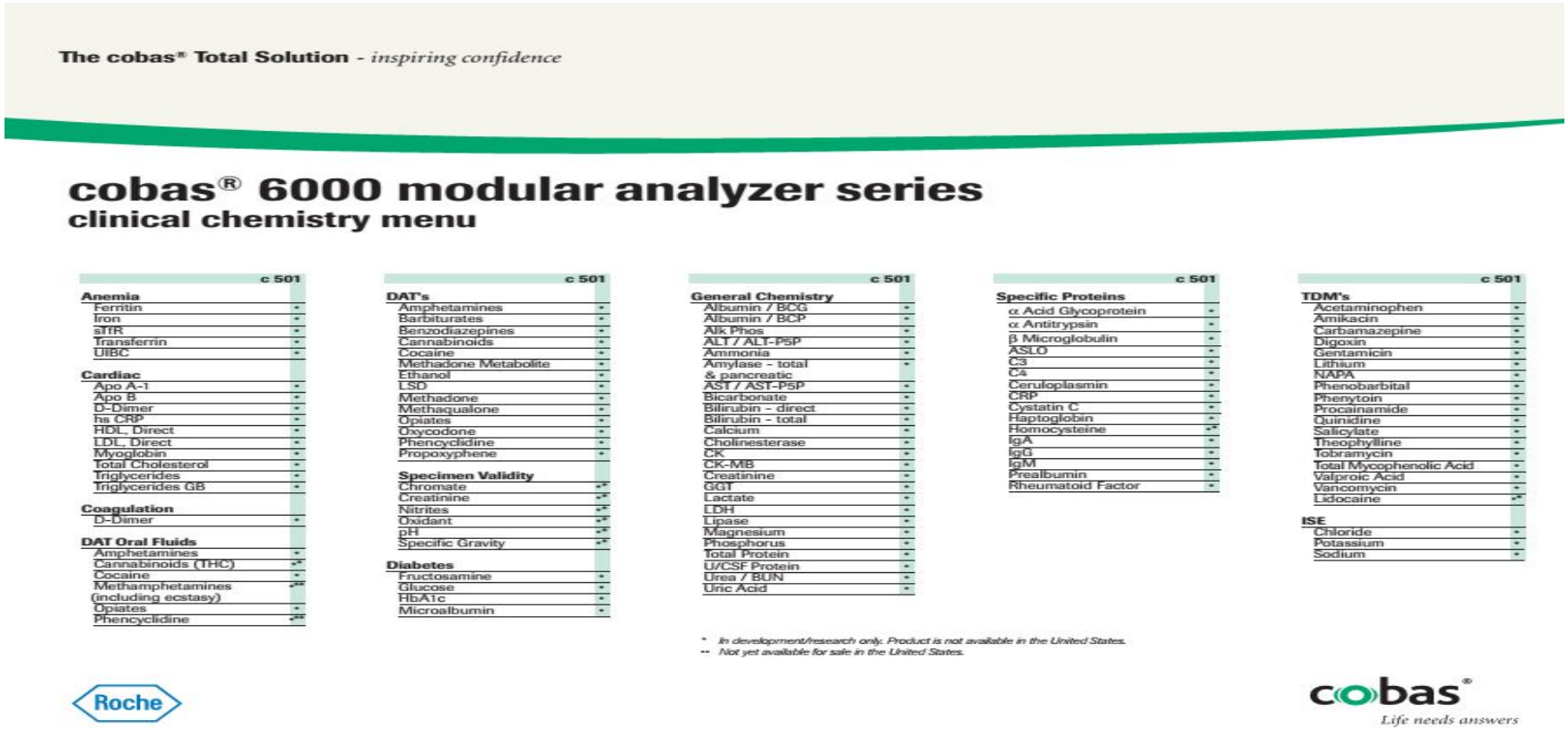
IPAC is a signatory to the EA MLA and ILAC MRA

O presente Certificado e o(s) seu(s) Anexo(s) Técnico(s) estão sujeitos a modificações, suspensões temporárias e eventual anulação. A sua actualização e validade pode ser confirmada na página www.ipac.pt.

This Certificate and its Annex(es) can be modified, temporarily suspended and eventually withdrawn. Its actualization and validity can be

Appendix E - Roche's Cobas® 6000 Tests

E.1 Cobas® 6000: module c501 list of tests



The cobas® Total Solution - *inspiring confidence*

cobas® 6000 modular analyzer series
clinical chemistry menu

	c 501
Anemia	
Ferritin	•
Iron	•
sTfR	•
Transferrin	•
UIBC	•
Cardiac	
Apo A-1	•
Apo B	•
D-Dimer	•
hs CRP	•
HDL, Direct	•
LDL, Direct	•
Myoglobin	•
Total Cholesterol	•
Triglycerides	•
Triglycerides GB	•
Coagulation	
D-Dimer	•
DAT Oral Fluids	
Amphetamines	•
Cannabinoids (THC)	•*
Cocaine	•
Methamphetamines (including ecstasy)	•**
Opiates	•
Phencyclidine	•**

	c 501
DAT's	
Amphetamines	•
Barbiturates	•
Benzodiazepines	•
Cannabinoids	•
Cocaine	•
Methadone Metabolite	•
Ethanol	•
LSD	•
Methadone	•
Methaqualone	•
Opiates	•
Oxycodone	•
Phencyclidine	•
Propoxyphene	•
Specimen Validity	
Chromate	•*
Creatinine	•*
Nitrites	•*
Oxidant	•*
pH	•*
Specific Gravity	•*
Diabetes	
Fructosamine	•
Glucose	•
HbA1c	•
Microalbumin	•

	c 501
General Chemistry	
Albumin / BCG	•
Albumin / BCP	•
Alk Phos	•
ALT / ALT-P5P	•
Ammonia	•
Amylase - total & pancreatic	•
AST / AST-P5P	•
Bicarbonate	•
Bilirubin - direct	•
Bilirubin - total	•
Calcium	•
Cholinesterase	•
CK	•
CK-MB	•
Creatinine	•
GGT	•
Lactate	•
LDH	•
Lipase	•
Magnesium	•
Phosphorus	•
Total Protein	•
U/CSF Protein	•
Urea / BUN	•
Uric Acid	•

	c 501
Specific Proteins	
α Acid Glycoprotein	•
α Antitrypsin	•
β Microglobulin	•
ASLO	•
C3	•
C4	•
Ceruloplasmin	•
CRP	•
Cystatin C	•
Haptoglobin	•
Homocysteine	•*
IgA	•
IgG	•
IgM	•
Prealbumin	•
Rheumatoid Factor	•

	c 501
TDM's	
Acetaminophen	•
Amikacin	•
Carbamazepine	•
Digoxin	•
Gentamicin	•
Lithium	•
NAPA	•
Phenobarbital	•
Phenytoin	•
Procainamide	•
Quinidine	•
Salicylate	•
Theophylline	•
Tobramycin	•
Total Mycophenolic Acid	•
Valproic Acid	•
Vancomycin	•
Lidocaine	•*
ISE	
Chloride	•
Potassium	•
Sodium	•

* *In development/research only. Product is not available in the United States.*
** *Not yet available for sale in the United States.*

Roche

cobas®
Life needs answers

E.2 Cobas® 6000: module c501 list of tests

cobas® 6000 modular analyzer series
immunoassay menu

Anemia	e 601
Ferritin	•
Folate	•
RBC Folate	•
Vitamin B12	•
Bone Markers	
Beta Crosslaps	•
Osteocalcin	•
PTH	•
Vitamin D	•**
Tumor Markers	
AFP	•
CA 125 II	•
CA 15-3 II	•
CA 19-9	•
CEA	•
Free PSA	•
Total PSA	•
HE4	•**
Diabetes	
C-Peptide	•
Insulin	•

Fertility/Hormones	e 601
ACTH	•
Cortisol	•
DHEA-S	•
Estradiol II	•
FSH	•
HCG+β	•
LH	•
PlGF	•*
Progesterone II	•
Prolactin II	•
sFLT1	•*
SHBG	•
Testosterone	•
Thyroid Function	
Anti-Tg	•
Anti-TPO	•
Anti-TSHR	•
FT3	•
FT4	•
T3	•
T4	•
TSH	•
T-Uptake	•
Thyroglobulin	•**

Cardiac Markers	e 601
CK-MB	•
Digoxin	•
Myoglobin	•
proBNP II	•
Troponin T	•
Troponin I	•
Troponin T hs	•*
Specific Proteins	
IgE II	•
Hepatitis	
Anti-HAV	•
Anti-HAV IgM	•
Anti-HBs	•
Anti-HCV	•
HBsAg	•
HBsAg Conf.	•
Anti-HBc	•*
Anti-HBc IgM	•
Growth Hormones	
hGH	•

Infectious Disease	e 601
CMV IgG	•*
CMV IgM	•*
HSV Type I & II	•*
Rubella IgG	•
Rubella IgM	•
Syphillis	•*
Toxo IgG	•
Toxo IgM	•*
Sepsis/Inflammation	
IL-6	•*
Procalcitonin	•*
Rheumatoid Arthritis	
Anti-CCP	•
Stat (9-Minutes)	
CK-MB	•
HCG	•
proBNP II	•
PTH	•
Myoglobin	•
Troponin I	•
Troponin T	•
Troponin T hs	•*

Immunosuppresant Drugs	e 601
Cyclosporine	•*
Sirolimus	•*
Tacrolimus	•*

For more information about the cobas modular platform, contact your Roche representative or call 1-800-346-8606.
For distribution in the United States only.

* *In development/research only. Product is not available in the United States.*
** *Not approved/cleared or available for use in the United States. A PMA/510K submission is pending.*

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250
www.mylabonline.com

Appendix F - Roche's Traceability and Uncertainty Certificate (Calibrator Uncertainty)

F.1 *Traceability and Uncertainty* - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche® (2011)

TRACEABILITY and UNCERTAINTY [7]

cobas c 501 / c 502 / c 311 / c 701 / c 702 - C.f.a.s.

Cat. No. 10 759 350 190

Roche

Roche Diagnostics GmbH

March 2011

Routine Method Roche / cobas c Systems	Reference Method	Reference Material	Selected Measurement Procedure	Calibrator Value	Uncertainty [1]	Unit
CHED2 Cholinesterase *butyrylthiocholine Gen. 2*			Roche reagent, manual measurement	3730 62.3	34.8 0.581	U/L µkat/L
CHOL2 Cholesterol *CHOD-PAP Gen. 2 stand. ID/MS*	ID-MS[4]			165 4.28	1.46 0.0377	mg/dL mmol/L
CHOL2 Cholesterol *CHOD-PAP Gen. 2 stand. Abell-Kendall*	Abell-Kendall			163 4.22	1.43 0.0370	mg/dL mmol/L
CKL Creatine kinase *IFCC liquid* **cobas c 501/ c 502 / c 311**	Original formulation IFCC[5] (2002), manual measurement			316 5.28	2.64 0.0441	U/L µkat/L
CK Creatine kinase *IFCC liquid* **cobas c 701/ c 702**	Original formulation IFCC[5] (2002), manual measurement			324 5.41	2.39 0.0399	U/L µkat/L
CREP2 Creatinine *plus ver. 2*	ID-MS[4]			3.82 338	0.0348 3.08	mg/dL µmol/L
CREJ2 Creatinine *Jaffé compensated Gen. 2 STAT serum, plasma*	ID-MS[4]			3.97 351	0.0643 5.69	mg/dL µmol/L
CREJ2 Creatinine *Jaffé rate-blanked and compensated Gen. 2 serum, plasma*	ID-MS[4]			4.06 359	0.0576 5.10	mg/dL µmol/L

F.2 *Traceability and Uncertainty* - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche® (2011)

TRACEABILITY and UNCERTAINTY [7]

cobas c 501 / c 502 / c 311 / c 701 / c 702 - C.f.a.s.

Cat. No. 10 759 350 190

Roche Diagnostics GmbH

March 2011

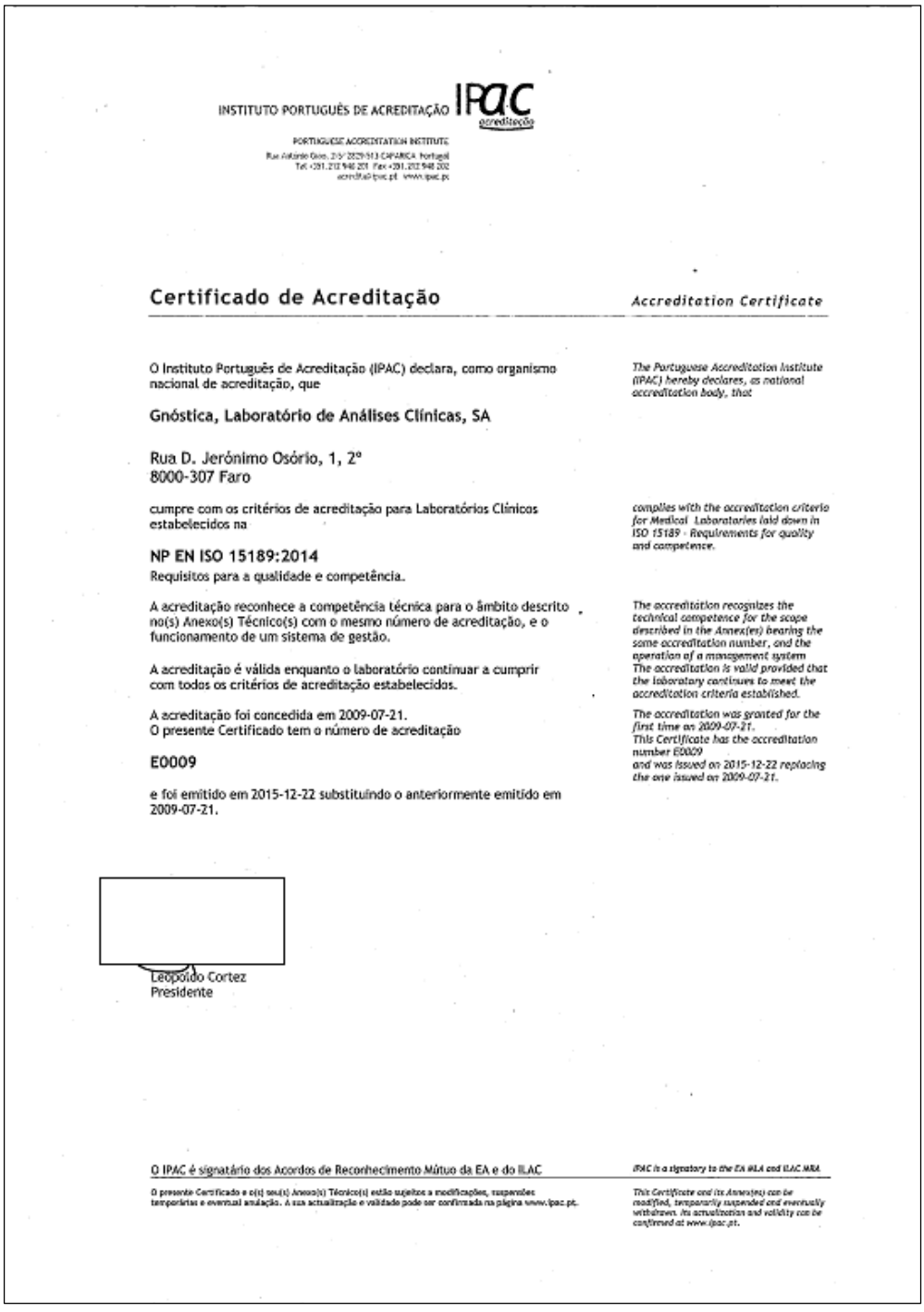
Routine Method Roche / cobas c Systems	Reference Method	Reference Material	Selected Measurement Procedure	Calibrator Value	Uncertainty [1]	Unit
GGT-2 Glutamyltransferase gamma *liquid stand. Szasz*	Original formulation Persijn/v.d. Slik (1976), manual measurement			91.8 1.53	1.25 0.0209	U/L µkat/L
GGT-2 Glutamyltransferase *gamma liquid stand. IFCC*	Original formulation IFCC[3] (2002), manual measurement			104 1.74	0.767 0.0128	U/L µkat/L
GLDH3 Glutamate dehydrogenase *opt. (DGKC) Gen. 3*			Roche reagent, manual measurement	21.9 0.366	0.370 0.00618	U/L µkat/L
GLUC2 Glucose *HK*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC2 Glucose *HK STAT*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC3 Glucose *HK*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC3 Glucose *HK STAT*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC2 Glucose ver. 2 *HK hemolysate Gen. 2*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC2 Glucose ver. 2 *HK hemolysate Gen. 2 STAT*	ID-MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L

cobas c systems_C f a s _Ver 8.doc

Page 6 of 10

Appendix G - Accreditation Certificate (Year2015)

G.1 Gnóstica Medical Laboratory - Accreditation Certificate: ISO 15189:2014 (IPAC - 2015/12/22)



INSTITUTO PORTUGUÊS DE ACREDITAÇÃO IPAC acreditação

PORTUGUESE ACCREDITATION INSTITUTE

Rua Antonio Gois, 2-5º 2829-513 CAPARICA Portugal
Tel. +351 212 948 251 Fax +351 212 948 252
acred@ipac.pt www.ipac.pt

Certificado de Acreditação — *Accreditation Certificate*

O Instituto Português de Acreditação (IPAC) declara, como organismo nacional de acreditação, que

The Portuguese Accreditation Institute (IPAC) hereby declares, as national accreditation body, that

Gnóstica, Laboratório de Análises Clínicas, SA

Rua D. Jerónimo Osório, 1, 2º
8000-307 Faro

cumpre com os critérios de acreditação para Laboratórios Clínicos estabelecidos na

complies with the accreditation criteria for Medical Laboratories laid down in ISO 15189 - Requirements for quality and competence.

NP EN ISO 15189:2014
Requisitos para a qualidade e competência.

A acreditação reconhece a competência técnica para o âmbito descrito no(s) Anexo(s) Técnico(s) com o mesmo número de acreditação, e o funcionamento de um sistema de gestão.

The accreditation recognizes the technical competence for the scope described in the Annex(es) bearing the same accreditation number, and the operation of a management system

A acreditação é válida enquanto o laboratório continuar a cumprir com todos os critérios de acreditação estabelecidos.

The accreditation is valid provided that the laboratory continues to meet the accreditation criteria established.

A acreditação foi concedida em 2009-07-21.
O presente Certificado tem o número de acreditação

The accreditation was granted for the first time on 2009-07-21.
This Certificate has the accreditation number E0009

E0009

e foi emitido em 2015-12-22 substituindo o anteriormente emitido em 2009-07-21.

and was issued on 2015-12-22 replacing the one issued on 2009-07-21.

Leopoldo Cortez
Presidente

O IPAC é signatário dos Acordos de Reconhecimento Mútuo da EA e do ILAC

IPAC is a signatory to the EA MLA and ILAC MRA

O presente Certificado e o(s) seu(s) Anexo(s) Técnico(s) estão sujeitos a modificações, suspensões temporárias e eventual anulação. A sua actualização e validade pode ser confirmada na página www.ipac.pt.

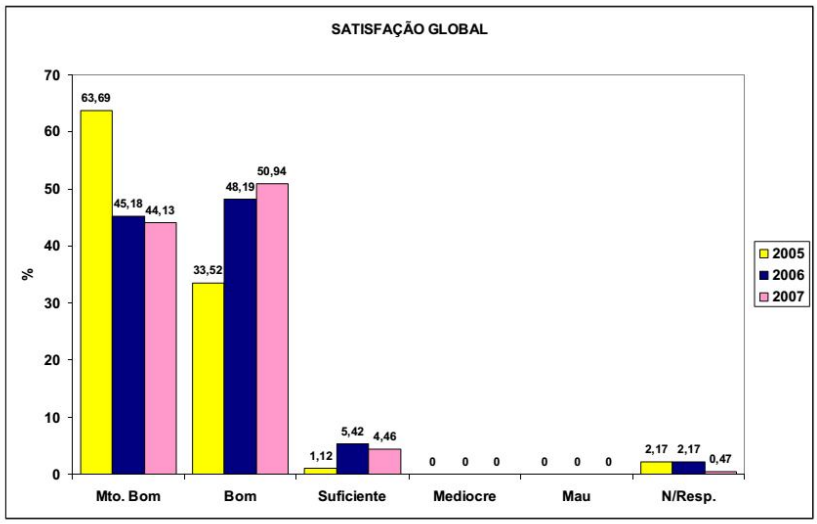
This Certificate and its Annex(es) can be modified, temporarily suspended and eventually withdrawn. Its actualization and validity can be confirmed at www.ipac.pt.

Appendix H - *Quality Indicators (6.1 Results)*

H.1 *User's Overall Satisfaction* (2005 – 2006 – 2007)

Enóstica - Laboratório de Análises Clínicas
Dr.ª Marília E. Sousa Faísca
Especialista em Análises Clínicas
Rua D. Jerónimo Osório, n.º 1 - 2.º
Telefone 289821002 8000-307 FARO
Fax 289801489

A Avaliação Global dos serviços subiu ligeiramente no sentido do Bom, reflectindo o esforço realizado em 2007 no sentido de diminuição das falhas e erros sobretudo ao nível da recepção, que se tinham verificado no ano anterior.

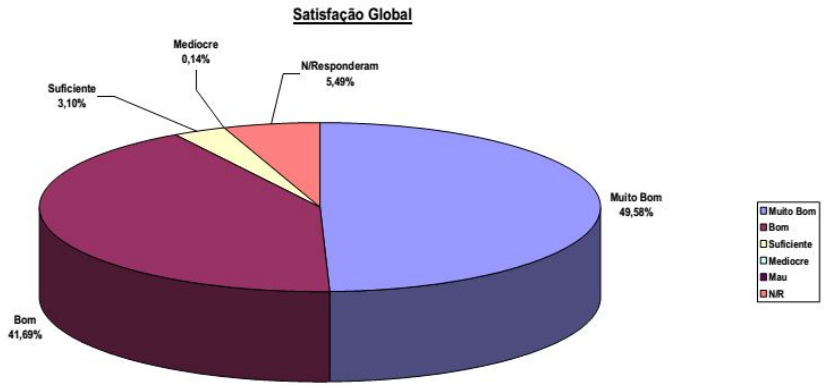


H.2 *User's Overall Satisfaction* (2009)

Gnóstica - Laboratório de Análises Clínicas
Dr.ª Marília E. Sousa Faísca
Especialista em Análises Clínicas
Rua D. Jerónimo Osório, n.º 1 - 2.º
Telefone 289821002 8000-307 FARO
Fax 289801489

No universo total obtivemos uma taxa de adesão de 50,7% (2008: 47%), relativamente maior que no ano anterior e muito mais abrangente em termos geográficos, de forma a reflectir melhor a opinião dos nossos utentes

Os resultados em termos de apreciação global foram os seguintes:



H.3 *User's Overall Satisfaction* (2010)

Laboratório de Análises Clínicas

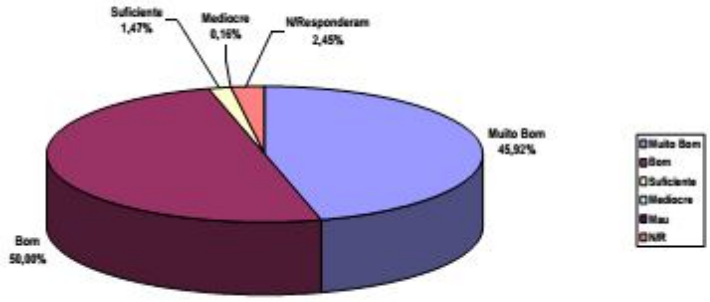
REVISÃO PELA GESTÃO - 2010

	Versão	Data	Paginação
RG-4-15	00	20-01-2011	Pag. 7 de 51

No universo total obtivemos uma taxa de adesão de 49,4% (2009: 50,7%), ligeiramente mais baixa que no ano anterior, mas abrangente em termos geográficos, de forma a reflectir melhor a opinião dos nossos utentes

Os resultados em termos de apreciação global foram os seguintes:

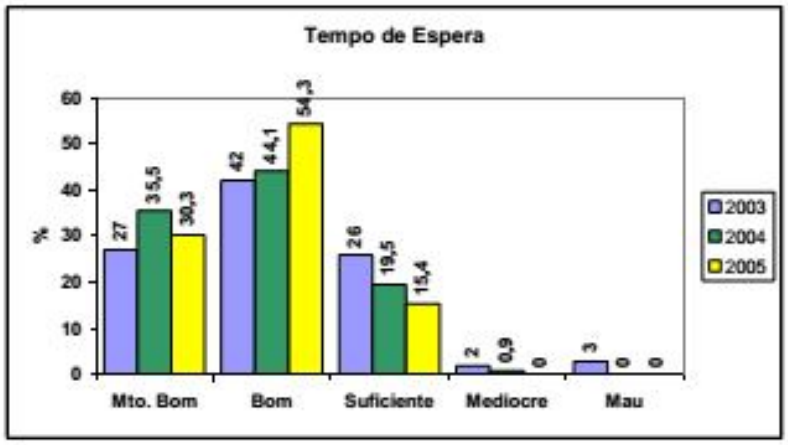
Satisfação Global



Appendix I - Quality Indicators (6.1 Results)

I.1 Pre-analytical Phase - Waiting Time (%) – 2003 - 2005

3.1.2. Avaliação do Tempo de Espera dos Utentes até serem Atendidos

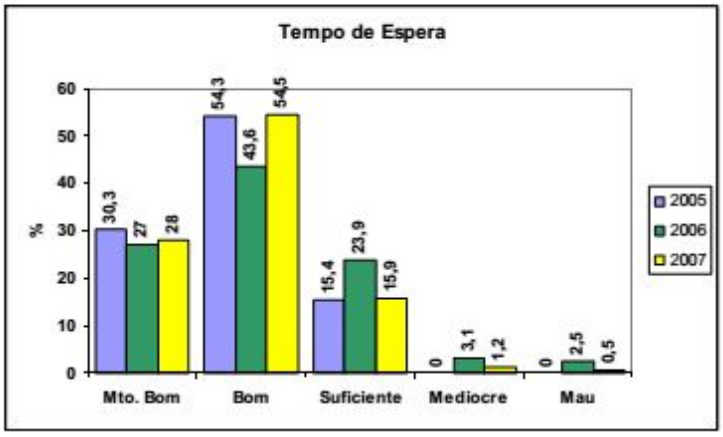


Apesar de todos os nossos esforços, de modo a melhorar esta apreciação, não foi possível, embora se observe uma subida no sentido do bom e simultânea descida do suficiente, o que revela alguns progressos na gestão do tempo durante o processo da colheita.

I.2 Pre-analytical Phase - Waiting Time (%) – 2005 - 2007

Gnóstica - Laboratório de Análises Clínicas
Dr.ª Marília E. Sousa Faísca
Especialista em Análises Clínicas
Rua D. Jerónimo Osório, n.º 1 - 2.º
Telefone 289821602 8000-307 FARO
Fax 289801489

4.1.2. Avaliação do Tempo de Espera dos Utentes até serem Atendidos



Devido ao número de utentes no laboratório central, é preciso ter sempre no mínimo, na recepção duas funcionárias treinadas e nas colheitas duas a três flebotomistas.
Esta premissa funciona, uma vez que a avaliação dos utentes melhorou substancialmente, assim como a avaliação estatística dos dados fornecidos informaticamente, que nos dá o tempo que medeia a hora de inscrição e a hora de colheita

Appendix J - Roche's Traceability and Uncertainty Certificate (Calibrator Uncertainty)

J.1 *Traceability and Uncertainty* - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche® (2014)

TRACEABILITY and UNCERTAINTY [7]

cobas c 501 / c 502 / c 311 / c 701 / c 702 - C.f.a.s.

Cat. No. 10 759 350 190

Roche Diagnostics GmbH — September 2014

Routine Method Roche / cobas c systems	Reference Method	Reference Material	Selected Measurement Procedure	Calibrator Value	Uncertainty [1]	Unit
Ca2 Calcium *nM-BAPTA STAT* **cobas c 701/ c 702**		SRM[3] 956c L2		2.73 10.9	0.0143 0.0573	mmol/L mg/dL
CHE2 Cholinesterase *butyrylthiocholine Gen. 2*			Roche reagent, manual measurement	3730 62.3	34.8 0.581	U/L µkat/L
CHE Cholinesterase *acethylthiocholine*			Roche reagent, manual measurement	2630 43.9	22.9 0.382	U/L µkat/L
CHED2 Cholinesterase *butyrylthiocholine Gen. 2*			Roche reagent, manual measurement	3730 62.3	34.8 0.581	U/L µkat/L
CHOL2 Cholesterol *CHOD-PAP Gen. 2 stand. ID/MS*	ID-MS[4]			168 4.34	1.70 0.0440	mg/dL mmol/L
CKL Creatine kinase *IFCC liquid* **cobas c 501/ c 502 / c 311**	Original formulation IFCC[5] (2002), manual measurement			355 5.93	2.37 0.0396	U/L µkat/L
CK Creatine kinase *IFCC liquid* **cobas c 701/ c 702**	Original formulation IFCC[5] (2002), manual measurement			329 5.49	1.96 0.0327	U/L µkat/L
CREP2 Creatinine *plus ver. 2*	ID-MS[4]			3.82 338	0.0348 3.08	mg/dL µmol/L

J.2 *Traceability and Uncertainty* - Cobas® c501 / c502 / c311 / c701 / c702 (C.a.s.f.), from Roche® (2014)

TRACEABILITY and UNCERTAINTY [7]

cobas c 501 / c 502 / c 311 / c 701 / c 702 - C.f.a.s.

Cat. No. 10 759 350 190

Roche Diagnostics GmbH — September 2014

Routine Method Roche / cobas c systems	Reference Method	Reference Material	Selected Measurement Procedure	Calibrator Value	Uncertainty [1]	Unit
CREJ2 Creatinine *Jaffé compensated Gen. 2 STAT* *serum, plasma*	ID-MS[4]			3.97 351	0.0643 5.69	mg/dL µmol/L
CREJ2 Creatinine *Jaffé rate-blanked and compensated* *Gen. 2 serum, plasma*	ID-MS[4]			4.06 359	0.0576 5.10	mg/dL µmol/L
GGT-2 Glutamyltransferase gamma *liquid stand. Szasz*	Original formulation Persijn/v.d. Slik (1976), manual measurement			91.8 1.53	1.25 0.0209	U/L µkat/L
GGT-2 Glutamyltransferase *gamma* *liquid stand. IFCC*	Original formulation IFCC[5] (2002), manual measurement			104 1.74	0.767 0.0128	U/L µkat/L
GLDH3 Glutamate dehydrogenase *opt. (DGKC) Gen. 3*			Roche reagent, manual measurement	21.9 0.366	0.370 0.00618	U/L µkat/L
GLUC2 Glucose *HK*	ID -MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC2 Glucose *HK STAT*	ID -MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L
GLUC3 Glucose *HK*	ID -MS[4]			193 10.7	1.63 0.0905	mg/dL mmol/L

Appendix K - Pre-analytical Uncertainty: Sample Analyte Data *(Mean Concentrations and CV's %)*

	Mean Concentration						*CV* (%)				
Test / Units	A) Reference Measurement	B) Blood Collection	B1) Refrigerated Samples	B2) Frozen Samples	C) Sample Processing	D) Regional Transportation	*B) Blood Collection*	*B1) Refrigerated Samples*	*B2) Frozen Samples*	*C) Sample Processing*	*D) Regional Transportation*
ALB */ g/dL*	4,66	*4,57*	*4,62*	*4,56*	*4,58*	*4,61*	***3,20***	***1,54***	***1,83***	***1,09***	***1,19***
ALP / *U/L*	61,21	*59,93*	*60,07*	*59,59*	*59,93*	*59,72*	***2,44***	***0,98***	***1,73***	***0,98***	***1,46***
ALT / *U/L*	18,50	*18,58*	*18,08*	*16,42*	*19,12*	*18,35*	***6,94***	***3,12***	***9,90***	***9,87***	***2,60***
AST / *U/L*	20,70	*20,97*	*20,97*	*20,70*	*20,80*	*21,07*	***3,16***	***2,30***	***4,96***	***3,44***	***2,68***
Ca */ mg/dL*	9,58	*9,42*	*9,36*	*9,25*	*9,35*	*9,32*	***1,90***	***2,23***	***3,13***	***1,26***	***1,01***
CK / *U/L*	132,80	*136,73*	*133,83*	*129,43*	*132,23*	*131,93*	***4,13***	***2,60***	***5,51***	***4,11***	***4,32***
Cl */ mmol/L*	101,14	*100,77*	*100,78*	*100,42*	*100,41*	*100,25*	***0,76***	***0,73***	***0,74***	***0,58***	***0,62***
GGT / *U/L*	26,67	*26,63*	*26,30*	*25,70*	*26,43*	*26,20*	***2,42***	***1,83***	***6,80***	***2,66***	***3,13***
HDL-C	50,90	*50,80*	*49,50*	*49,60*	*50,77*	*50,73*	***1,63***	***2,40***	***4,10***	***0,98***	***1,02***
K */ mmol/L*	4,27	*4,27*	*4,34*	*4,24*	*4,25*	*4,38*	***3,74***	***3,25***	***3,10***	***1,51***	***2,84***
LDH / *U/L*	323,57	*331,70*	*305,27*	*318,13*	*338,27*	*343,83*	***4,44***	***8,18***	***5,68***	***3,45***	***5,15***
Mg */ mg/dL*	2,05	*2,03*	*2,06*	*2,04*	*2,05*	*2,03*	***1,20***	***2,29***	***2,59***	***1,21***	***4,51***
Na */ mmol/L*	141,00	*139,80*	*140,13*	*139,33*	*140,03*	*139,70*	***0,91***	***0,45***	***0,69***	***0,58***	***0,62***
TBIL */ mg/dL*	0,60	*0,58*	*0,53*	*0,54*	*0,56*	*0,54*	***4,60***	***8,89***	***7,48***	***7,46***	***8,07***
TP */ g/dL*	7,17	*7,06*	*7,11*	*7,04*	*7,07*	*7,08*	***1,95***	***1,33***	***2,12***	***0,84***	***0,80***
TG */ mg/dL*	137,43	*134,70*	*138,70*	*136,50*	*135,20*	*133,87*	***2,44***	***2,28***	***1,95***	***1,11***	***5,20***

Appendix L - Pre-analytical Uncertainty Components *(Combined and Expanded Pre-analytical Uncertainty)*

Test / Analyte	CV_A (%)	***Standard Uncertainty of*** *B) Blood Collection* **(uB %)**	*B1) Refrigerated Samples* **(uB1 %)**	*B2) Frozen Samples* **(uB2 %)**	*C) Sample Processing* **(uC %)**	*D) Regional Transportation* **(uD %)**	**Combined Preanalytical Uncertainty** $u_{c\,pre}$ *(%)*	**Expanded Preanalytical Uncertainty** U_{pre} *(%)*
ALB	1,93	**1,27**	< CV_A	< CV_A	< CV_A	< CV_A	**1,27**	**2,54**
ALP	2,17	**0,27**	< CV_A	< CV_A	< CV_A	< CV_A	**0,27**	**0,55**
ALT	1,84	**5,10**	**1,28**	**8,06**	**8,03**	**0,76**	**12,56**	**25,11**
AST	2,18	**0,98**	**0,12**	**2,78**	**1,26**	**0,50**	**3,24**	**6,49**
Ca	1,49	**0,41**	**0,74**	**1,64**	< CV_A	< CV_A	**1,85**	**3,70**
CK	1,30	**2,83**	**1,30**	**4,21**	**2,81**	**3,02**	**6,66**	**13,33**
Cl	1,30	< CV_A	< CV_A	< CV_A	< CV_A	< CV_A	**< CV_A**	**< CV_A**
GGT	2,37	**0,05**	< CV_A	**4,43**	**0,29**	**0,76**	**4,51**	**9,01**
HDL-C	1,95	< CV_A	**0,45**	**2,15**	< CV_A	< CV_A	**2,20**	**4,39**
K	1,21	**2,53**	**2,04**	**1,89**	**0,30**	**1,63**	**4,11**	**8,23**
LDH	1,50	**2,94**	**6,68**	**4,18**	**1,95**	**3,65**	**9,38**	**18,75**
Mg	1,59	< CV_A	**0,70**	**1,00**	< CV_A	**2,92**	**3,17**	**6,33**
Na	1,12	< CV_A	< CV_A	< CV_A	< CV_A	< CV_A	**< CV_A**	**< CV_A**
TBIL	3,96	**0,64**	**4,93**	**3,52**	**3,50**	**4,11**	**8,14**	**16,28**
TP	1,27	**0,68**	**0,06**	**0,85**	< CV_A	< CV_A	**1,09**	**2,17**
TG	1,60	**0,84**	**0,68**	**0,35**	< CV_A	**3,60**	**3,78**	**7,56**

Appendix M - Accreditation Auditing Report

M.1 Gnóstica's Accreditation Report (IPAC 2016)

IPAC acreditação

RELATÓRIO DE AVALIAÇÃO • Laboratórios • FOLHA DE ROSTO

OIA036 • 2012-01-31 **(12772)**

IDENTIFICAÇÃO DA AVALIAÇÃO

Entidade & Unidade(s) & Local	Nº Registo	Tipo de Avaliação	Datas
Gnóstica, Laboratório de Análises Clínicas, SA			2016-03-03
Gnóstica	E0009	Renovação	e: 2016-03-04
8000-307 Faro			

IDENTIFICAÇÃO DA EQUIPA AVALIADORA

RELATÓRIO

Introdução

Este Relatório descreve as conclusões e constatações feitas pela Equipa Avaliadora no decorrer da Avaliação, sendo constituído pelos documentos indicados abaixo.

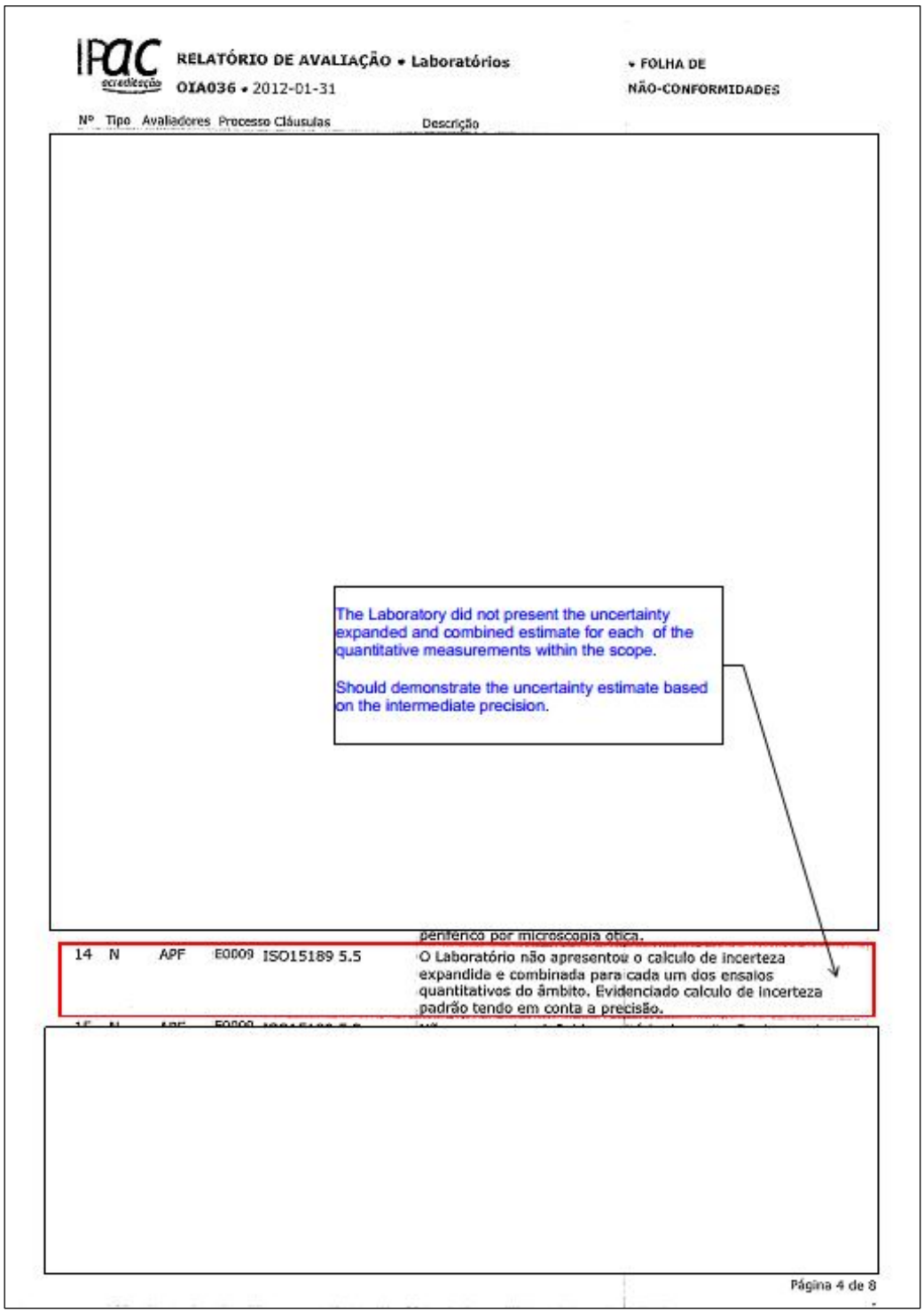
A Equipa Avaliadora chama a atenção para o facto de a Avaliação ser uma situação de amostragem, pelo que podem existir outras falhas, relacionadas ou não com as descritas neste Relatório, competindo à Entidade completar esta amostragem e implementar os mecanismos correctivos e preventivos necessários. Ressalva-se ainda que a Avaliação apenas incidiu sobre o âmbito descrito na(s) Folha(s) de Referencial da(s) página(s) seguinte(s).

Este Relatório, bem como toda a informação a que a Equipa Avaliadora teve acesso pela sua participação na Avaliação, será tratado de forma confidencial, não podendo ser reproduzido para terceiros sem autorização expressa do IPAC.

Documentos Anexos ao Relatório Nº Total de Anexos: 0 (0 Ficheiros)

Data de entrega de cópia do Relatório à Entidade Avaliada: 2016-03-04

M.2 Gnóstica's Accreditation Report (IPAC2016)



IPAC acreditação

RELATÓRIO DE AVALIAÇÃO • Laboratórios
OIA036 • 2012-01-31

• FOLHA DE NÃO-CONFORMIDADES

Nº	Tipo	Avaliadores	Processo	Cláusulas	Descrição
14	N	APF	E0009	ISO15189 5.5	periférico por microscopia ótica. O Laboratório não apresentou o calculo de incerteza expandida e combinada para cada um dos ensaios quantitativos do âmbito. Evidenciado calculo de incerteza padrão tendo em conta a precisão.

The Laboratory did not present the uncertainty expanded and combined estimate for each of the quantitative measurements within the scope.

Should demonstrate the uncertainty estimate based on the intermediate precision.

Página 4 de 8