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Quality Control in Laboratory

Edited by Gaffar Sarwar Zaman

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Notice

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Meet the editor

Dr. Zaman obtained the title of Doctor of Medicine in Biochemistry (MD) from Assam Medical College, Dibrugarh University, India; a Fellowship in Diabetes (FID) from the Royal Liverpool Academy, United Kingdom; a Fellowship in Applied Nutrition (FIAN) from Medvarsity, Apollo Hospitals, India; and a Post Graduate Diploma in Clinical Research (PGDCR) from Symbiosis International University, India. He has almost 12 years of experience as an Assistant Professor at the King Khalid University and Rajiv Gandhi University of Health Sciences.

He has experience in quality development and can help any college with the improvement of quality. He is also well accustomed in curriculum designing and is trained in e-learning methods. He has almost 40 research publications (most of them original articles) to his credit, in both national and international journals. Currently, he is also engaged in editing three books for IntechOpen.

Contents

Preface

Quality control (QC) is the most important component of any hospital or laboratory project. Clinical laboratory science has undergone a vast change in the last 25 years. Although a good design with properly prepared drawings and specifications is essential for a quality end product, the QC and quality assurance (QA) efforts are also essential to assure that the construction complies with the drawings and specifications. Various different reports on medical/laboratory fallacy/misconception have raised the visibility for the necessity to augment patient safety and quality resourcefulness. Prominence has relocated from simply diagnosing and treating disease to identifying and controlling disease, risk factors and maintaining health. The purpose of establishing laboratory quality standards is to ensure the accuracy of test results; to increase the confidence of patients, clinicians and communities in the value of laboratory testing; and to elucidate in patient management. Quality standards are an integral part of the quality system. They are designed to help laboratories meet regulatory requirements, including local health regulations, and monitor laboratory functions, thereby ensuring laboratory safety and consistency of performance. The purpose of this book is to provide procedures, guidance, references, checklists and worksheets to help in the quality control (Internal Quality Control and External Quality Assurance), quality management and QA and to help in performing periodic quality assurance inspection. The book stresses prominence on three facets: (1) components such as cases and controls, defined and well-managed processes, and integrity and performance aspects, (2) competence and (3) quality of laboratory reports. Each administrative and technical procedure in the laboratory must be subject to a quality monitoring process developed with the help of relevant laboratory staff. Each process must be documented using a step-by-step approach. Heads of laboratories are responsible for ensuring that each document is understood, and all processes are fully implemented by laboratory staff. Advances in technology have totally changed the way we practise diagnosis and treatment. Now, the testing techniques have moved from test tubes, beakers and large, automated analysers to microanalytic systems ("lab on a chip") that allow reduced sample size, fewer reagents and smaller instruments. Nowadays, patients can even carry small-sized instruments with them that can help them in finding out various parameters. The continuation, prolongation and preservation of a quality management system are crucial to a laboratory for providing the correct test results every time. All these, however, require rigid quality control. Establishing and maintaining laboratory quality standards are essential. They are important for several reasons, including ensuring the quality and traceability of patients' results, supporting clinical and public health decision-making, procuring equipment, using standard techniques and reagents, sharing documentation, training programmes, quality assurance, meeting requirements for reimbursement for national insurance schemes, and complying with national or international accreditation and licencing systems. A well-staffed and well-financed organization centre is critical for estab-

lishing and running a successful quality assurance programme, that supports participants. Hence, I sincerely hope that this book will enable all aspects of medical science and the patients to improve their everyday life.

This work would not have been possible without the support of IntechOpen. I am especially indebted to Ms. Romina Rovan, Publishing Process Manager, who has been supportive of my book and who worked actively to provide me with all facilities. I am grateful to all of those with whom I have had the pleasure to work during this project. Each of the authors of the different chapters has provided me extensive professional help and obliged me on this scientific venture wherever possible. I would especially like to thank the authors of the various chapters of the book:

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Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, Late Taufiquz Zaman and Late Jaiba Zaman, whose love and guidance are with me in whatever I pursue. Most importantly, I wish to thank my loving and supportive wife, Mrs. Jarin Tanwir Hussain, who is my ultimate role model, and my two wonderful children, Naushad Muntasir Zaman and Umar Sarwar Zaman, who provided me with unending inspiration during the project.

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Chapter 1

Introductory Chapter: History and Scope of Quality Control in Laboratories

Gaffar Sarwar Zaman

Additional information is available at the end of the chapter

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1. Introduction

Laboratory quality control encompasses the integral and essential monitoring of investigative analytics to find out investigative and interfering flaws, miscalculations and blunders that appear or materialize during the analytical procedures and conclusively avert the erroneous reporting of results to the patient. Quality control is usually used to monitor the accomplishment of a test, and, to find the accuracy. Levey and Jennings were the pioneers of using statistics for the standardization and calibration of analytical procedures. The substances used for quality control are known as quality control (QC) materials. They are usually aliquoted in a stable form. Nowadays, most laboratories purchase the QC material from reputed companies instead of making them. The QC material is usually in a powdered form and can be stored for more than a year. The concept of statistical quality control decreased to a great extent the cost of quality control by the help of the method of sampling.

2. Historical perspective of quality control in laboratories

Walter Andrew Shewhart (March 18, 1891–March 11, 1967) was an American engineer, statistician and physicist, also called "the Father of Modern Quality Control," and he is also credited as the founder of the "Shewhart cycle" [1] (**Figure 1**). Walter Shewhart was instrumental in the introduction and development of "Process Control" in the year 1924. Prevention of the manufacture of defective products was the main aim of this method. For

Figure 1. Walter Andrew Shewhart (March 18, 1891–March 11, 1967) [reference: http://www.york.ac.uk/depts/maths/histstat/people/shewhart.gif] [4].

Figure 2. William Edwards Deming (October 14, 1900–December 20, 1993). http://www.fda.gov/oc/initiatives/criticalpath/stanski/stanski.html [5].

this, he also suggested and developed control charts, known as "Average Shewhart Chart" and "R Shewhart Chart or Range Shewhart Chart". W. Edwards Deming was another pioneer in this field [2–5] (**Figure 2**). He developed 14 rules, which comprised of a series of successive steps of testing. His contributions boosted Japan's rapid industrial growth in the post-war period.

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2.1. Levey-Jennings chart

It is a chart in which the data from a quality control are plotted and from which visually we can find out whether a particular laboratory test is working or not. The name of the chart is given after Stanley Levey and Elmer R. Jennings who first introduced this chart in the 1950s. It has become so popular that nowadays that it is even used for automated analyzers.

When Rausch and Freier introduced that serum pools should be used in place of samples from patients, the chart of Stanley Levey and Elmer R. Jennings became even more popular. Thus, these samples that came to be called as "standards" were ultimately known as "control samples" [6].

In 1954, E.S. Page of the University of Cambridge introduced the sequential analysis technique known as the cumulative sum control chart (CUSUM). CUSUM was devised as a method to find out changes in the techniques or quality and also the exact time as to when to take corrective actions. But the CUSUM chart was only analyzed for feasible use in the laboratory many years later. It was followed by the invention of the "Exponentially Weighted Moving Average (EWMA)" chart in 1959 by the American S.W. Roberts [7]. However, the EWMA chart was adapted for use in medical laboratory applications much later [8].

However the use of patient results for quality control only started during 1960–1970. One of the pioneers in this field was the Japanese statistician Kaoru Ishikawa. Quality control was used for patients' tests involving hematology and biochemistry laboratories [9]. Dennis Dorsay, in 1963, was one of the pioneers who stressed the importance of erythrocyte indexes for quality control in various hematology analyzers [10].

Repeated assaying of whole blood samples from two successive days was advocated by Frank Ductra in 1966; this was to be done in place of control samples and also revolutionized the quality control process [11].

In 1965, Michael Waid and Robert Hoffmann introduced the unique "Average of normals" (AON) method where systematic errors can be detected by the arithmetic average of normal test results produced by biochemical analyses [12].

Quality control in hematology analyzers, called "Bull's algorithm" or "X_{-B}", was introduced in 1974 by the American hematologist Brian Bull [13].

The introduction of "Computer simulations" brought about a big change in the issues of quality control. It was introduced by the Swedish clinical engineer Torsten Aronson, medical doctor Carl-Henric de Verdier and a physicist Torgny Groth [10, 14]. In the same year, Arthur Gottmann and Jerome Nosanchuk [13, 14] utilized the new method of comparing each patient's results with previous results, within a specified time, to detect any errors made my analyzers. It proved to be a reliable and cost-effective quality method. The distinctive feature of this method was the use of patient's results instead of control samples, and it does not make any discrimination between normal and pathological values.

The "delta check method" (in comparison with the previous record) and the "rate check method" in which the time elapsed between measurements were being considered and were suggested by Nosanchuk and Gottmann [15]. Usefulness of the moving average was elucidated by the Canadian clinical chemist George Cembrowski and the American clinical pathologist James

Westgard in 1975 (**Figure 3**). A year after, the use of anion gap equation for automated blood gas and electrolyte analyzers quality control was advocated [16] by David Witte and co-workers.

"A multi-rule Shewhart Chart for quality control in clinical chemistry" was published during the 1980s by Westgard, marking a major breakthrough in quality control for laboratories. The simple rules explaining implementation of the Levy-Jennings chart were given in this chapter.

The initial international quality standard for operations in a clinical laboratory was also established during the 1980s. During the 1990s, the theoretical and practical application of biological variances as analytical targets in clinical chemistry [18, 19] were worked upon by Fraser and his co-workers, distinguishable among them being Eugene Harris, the American clinical chemist, who was instrumental in contributing to the formulation of the theory of biological variances through his expertise of statistics and informatics [20]. Another notable contribution is that of Carmen Ricos [21–23] and her group of Spanish researchers (majority), who were responsible for collecting data on quality specifications and biological variances a number of biochemical parameters.

The "OPSpecs charts" [24] concept was proposed by Westgard in 1994. Non-analytical errors, that is, errors that occur before or after analysis, were also discussed extensively during the 1990s. Configuration of laboratory information systems (LISs) led to the prevention of post-analytical errors and some types of pre-analytical errors.

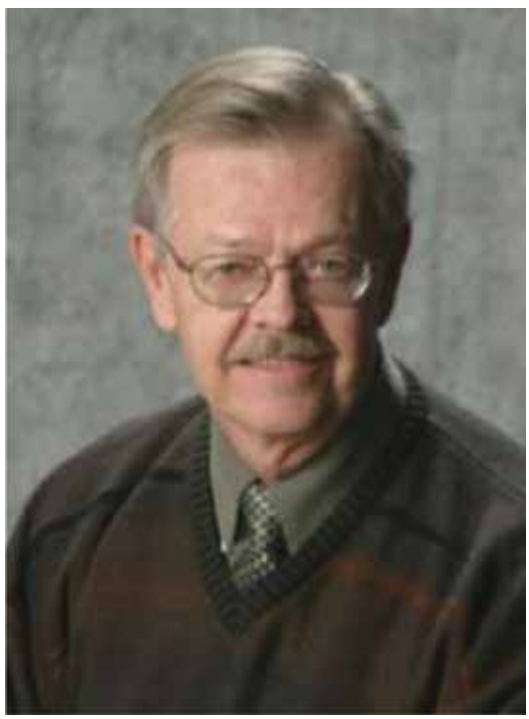

Figure 3. Professor James O. Westgard is President of Westgard QC, Inc., a small business providing education and training for laboratory quality management. He is an Emeritus Professor in the Department of Pathology and Laboratory Medicine at the University of Wisconsin Medical School [reference: https://www.labqualityconfab.com/speakers/james-o-westgard] [17].

Later on, Westgard introduced the six sigma theory in clinical chemistry, which proved to be another method of establishing quality specifications [25].

3. Salient features

The QC should proceed through three parts, mainly:

1. Each analytic method should have its own statistical limits of variation.
2. These limits should be utilized for finding out the QC data which is generated for each type of test.
3. Elimination of the various errors, and if found:

   (I) The cause of the error should be found out.

   (II) Action should be taken to correct the error.

   (III) The patients' data should be re-analyzed.

Multirule procedure: this includes decision criteria to determine if an analytic run is in control; it is used to detect random and systemic error over time and is developed by Westgard and Groth [26].

Proficiency testing, internal quality control, laboratory inspections, clinical utilization and quality assurance monitoring play an important role as indicators of analytic performance. Management of quality consists of quality design, quality control and quality improvement [26] (**Figure 4**).

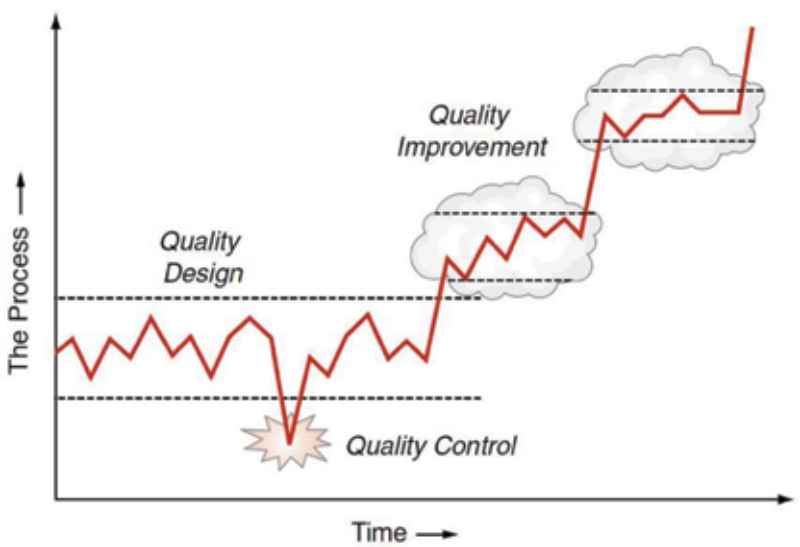

Figure 4. Relation between various aspects of quality control [26].

Use of automated analyzers in clinical laboratories: nowadays, almost every laboratory uses automated analyzers. The reason is that they are more reliable, can process more samples at a time, and are time saving and also cost saving in the long run. Most companies provide the quality control material along with the quality control guide. This has made it easier for laboratories to assess quality of the various types of parameters.

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Chapter 2

The Basic Concepts of Quality Control Reference: Interval Studies, Diagnostic Efficiency, and Method Evaluation in Quality Control

Ayed Dera

Additional information is available at the end of the chapter

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Abstract

Laboratory data are very important in making majority of the patient's decisions. Before introducing a new test to the patients, it is very important that the acceptable performance of the test is carried out. Hence, "method evaluation" should be carried out to find out and verify the accuracy of a new test before it is used in patients. Once the method has been approved, it is the job of the laboratory personnel to utilize "quality control" techniques to maintain it. All these fall under the system of "quality management." Laboratorians use the concepts of "descriptive statistics" for comparing and analyzing different data. Descriptive statistics encompasses a variety of measures. Diagnosis in the medical field and initiation and management of various therapies depend upon the comparison of the patient's test result with a "reference interval." A specified percentage of the values for a population is used to set the lower and upper reference limits. Reference interval should be established and verified before it can be used in patients. After establishing the reference interval, the analytic and pre-analytic variables must also be standardized in order to verify and make validations of that particular reference interval. There are numerous requirement establishment of a reference interval. Establishment of reference interval requires data analysis. A number of parameters are used to find out how efficient a particular test is for predicting or nullifying a particular disease. These parameters fall under the broad heading of "diagnostic efficiency." Diagnostic efficiency encompasses "predictive values," "specificity," and "sensitivity." It is very important that accurate and reliable test results are provided by the clinical laboratory service. To enable this, a method undergoes the full process of "method evaluation." "Imprecision" and "inaccuracy" are the first estimates to be made in a method evaluation; then, they are compared with the maximum allowable medical criteria-based error. Then, the use of "quality control" and "quality control charts" follows.

Keywords: method evaluation, quality control, quality management, descriptive statistics, reference interval, diagnostic efficiency, predictive values, specificity, sensitivity, imprecision, inaccuracy

1. Introduction to method evaluation and quality management

The current nature of conducting medical transactions and procedures has revealed that most of the underlying medical decisions are arrived at utilizing laboratory data. As a result, there is the great significance that the outcomes emanating from the laboratory be of the high degree of accuracy. Determination and upholding of accuracy call for considerable cost and potential, involving the utilization of several approaches in accordance with the underlying test's complexity [1]. Invariably, commencing the entire decision-making process, one is entitled to acknowledge the necessary quality besides knowing how to measure the quality. In conjunction with that, there are several statistical techniques deployed to enable the medical practitioner to measure the resultant quality. Prior to enacting a modern test, there is the essence of determining whether the test can be pursued acceptably wherein method evaluation is deployed in verifying the acceptability that accrues to the new approaches before reporting the results to the patient. Immediately, an approach has been enacted, a necessity prevails regarding that the laboratory ensures its validity over time. Quality control is the process that facilitates the upholding of the validity accruing to the laboratory over time. All the two concepts—method evaluation and quality control—are effective constituents of quality management. Invariably, quality management entails that the aggregate testing process is directed to the chief goal of enhancing the accuracy that accrues to the laboratory results [2]. This chapter presents the basic statistical concepts besides providing a universal overview regarding the procedures crucial for enacting a new method to ensure its persistent accuracy.

2. Basic concepts of quality control

On a daily basis, too many clinical laboratories prove to generate a wide range of results. This pool of clinical lab data ought to be summarized with an aim of monitoring the test performance. The basis for tracking performance—the quality control—is descriptive statistics, which involves three key concepts: measures of spread, shape, and center.

2.1. Descriptive statistics: measures of spread, shape, and center

After close examination, a combination of nearly identical aspects typically exhibits at least some differences for a certain property like smoothness, color, potency, volume, weight, and size. Likewise, laboratory data will possess at least some measurement differences. An effective example entails that if the glucose present in a specimen is examined a hundred times in one row, then there would emerge a range of the resultant data wherein such differences in the lab values can affect outcomes of several sources. Despite the fact that measurements differ, their resultant values yield patterns whose visualization and analysis can prevail collectively.

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The laboratorians describe and perceive these patterns deploying graphical representations as well as descriptive statistics. Nevertheless, once comparing and also analyzing sets of lab data, the description of the patterns can occur focusing on their spread, shape, and center. Even though the comparison of the data's center is quite typical, comparison of the spread is fairly more powerful. Nonetheless, data dispersion enables the lab practitioners to evaluate the predictability, as well as the lack of, in the lab test or rather a measurement.

2.2. Measures of center

The three typically deployed descriptions regarding the center include the mode, the median, and the mean. The mean is sometimes termed as the average of various data values. The median encompasses the "middle" point accruing to the data and is frequently deployed with fairly skewed data. The mode encounters its use rarely in describing the center of data but is often utilized in describing the data that deems to have two centers or rather bimodal data. The mean of the lab data can be acquired by summing up the total data values and dividing by the total number of samples or objects (**Figure 1**). Computing the median necessitates arrangement of the data values as per their ranks—either in an ascending manner or descending manner. Two values dominate the middle of the data, and then the median is an average of the two middle values. On the other hand, the mode entailed the most frequently appearing data value in the underlying dataset. It is often deployed in conjunction with the data's shape, bimodal distributions.

2.3. Measures of spread

The spread of the data depicts the distribution of the various data values. The spread further denotes the correlation of the entire data points to the data's mean. The descriptions of spread include standard deviation (SD), range, and coefficient of variation (CV). The range simply refers to the largest value regarding the dataset minus the dataset's smallest value. It denotes the data's extreme that one may identify standard deviation is a frequently deployed approach, especially when measuring variation. The SD and the variance denote the "average" distance notably from the data's center (mean) to every other value in the underlying dataset.

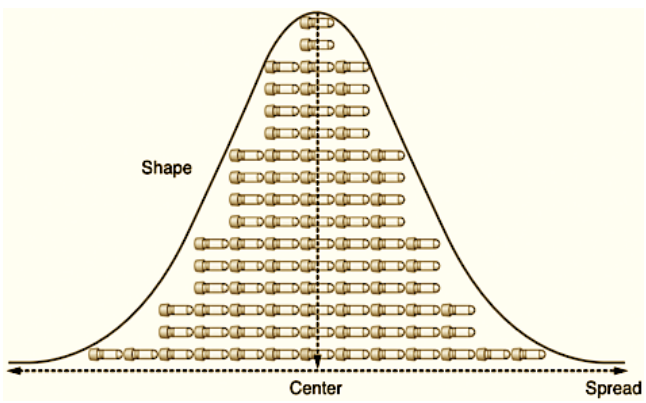

Figure 1. Basic measurements of data include the center, spread, and shape [1].

Furthermore, the CV enables the laboratorians to put up an effective comparison regarding the SDs with varying units. Computation of a dataset's SD necessitates prior computation of the dataset's variance (s^2). Variance precisely implies the average accruing to the squared distances of all the dataset's values from the set's mean. Variance, as a dispersion measure, denotes the difference dominant between each data value and the data's average. Afterward, the SD is simply the variance's square root. An additional approach of connoting SD is using the CV, which is computed via division of the SD by the mean of the data, and multiplying the quotient by 100 to represent it as a percentage (**Figure 1**). The CV proves to simplify the comparison of SDs accruing to test outcomes connoted in varying concentrations and units. The CV encounters extensive application in summarizing the underlying QC data, and it can be less than 1% for the highly precise analyzers.

2.4. Measures of shape

The most prevalent shape distributions accruing to datasets include the normal distribution (or the Gaussian distribution). This distribution proves to describe many lab variables that are continuous besides sharing various unique properties—the mode, median, and mean are identical. This distribution is further symmetric—since half of the values dominate the left side of the mean, whereas the other half is on the right side of the mean value. The symmetrical shape normally encounters the perception of being a "bell curve." The aggregate area covered by the Gaussian curve totals to 1.0 or rather 100%. Precisely, selecting a value in a Gaussian distributed dataset reveals that there is a 68% probability of finding the value between ±1 SD and the mean value. Likewise, there is 95% likelihood of finding the value between ±2 SDs and the mean value. There is further 99% probability of finding the value between ±3SDs and the mean value of the dataset (**Figure 1**). Universally, plotting patient data in histograms makes it a simple approach to visualize the underlying distribution of the dataset. Nonetheless, one can as well perform other mathematical analyses like normality tests to affirm whether data fits into a certain distribution.

3. Descriptive statistics for groups of paired observations

COM (comparison of method) is common for laboratorians dealing with data for many patients per unit time. A COM examination entails evaluating the patient's specimens by a reference (existing) technique and a test (new) approach. The resultant data from such comparisons encompass two measurements accruing to each of the patient's specimen. Convention enables plotting of the values acquired via the reference approach on the x-axis, whereas the values yielded by the test approach dominate the y-axis. Nevertheless, linear regression is a statistical approach whose analysis offers objective measures accruing to the dispersion and location of the best fit line. A linear regression yields three aspects—the y-intercept, the correlation coefficient, and the slope. The sign of the correlation coefficient indicates the relationship between the two plotted variables, and a higher coefficient indicates the prevalence of a splendid agreement notably between the comparative methods and the test [3, 4].

The difference plot, also called the Bland–Altman plot, is an additional approach regarding visualization of paired data. This approach graphs the absolute bias or even the percent bias

(difference) prevalent between the test method and the reference approach values divided by the range of the dataset. The difference plot further enables simple comparison regarding the differences in order to previously set up maximum limits [1]. Invariably, the main difference between reference and test method depicts the underlying error. COM experiments have a correlation with prevalence of two types of errors—systematic errors and random errors. The random errors are dominant in nearly all measurements besides being either negative or positive. Random error can emanate from environmental variations, an instrument used, reagent, and operator variations. Computation of the random error calls for calculation of the dataset's SD regarding the regression line. This error implies the average distance notably between the regression line and the data. A larger random error implies a wider scattered data values. Nevertheless, if the data points were perfectly in the same alignment as the regression line, the dataset's random error or rather the standard error would be zero. On the other hand, the systematic error affects observations in a consistent manner and also in one direction. The measures of y-intercept and slope yield an estimate regarding the systematic error. Invariably, systematic error can encounter categorization into proportional and constant errors. The constant systematic errors prevail once a continual difference exists between the test approach and the underlying comparative technique values, irrespective of the dataset's concentration. A proportional error prevails once the differences accruing to the test approach and the comparative approach values are fairly proportional to the underlying analyte concentration. Whenever the slope is not equal to one, a proportional error is present in that dataset.

4. Inferential statistics

Inferential statistics is the subsequent degree of complexity past paired descriptive statistics. They are deployed in drawing conclusions or rather inferences convening the SDs or mean of two datasets. Nevertheless, inferential statistics acknowledges the relevance of data distribution regarding shape. The respective distribution is key in determining the type of inferential statistics to use in analyzing the underlying data. Data depicting Gaussian distribution is normally analyzed deploying "parametric" tests that encompass ANOVA (a Student's t-test or analysis of variance). "Nonparametric" analysis is used for the data that is not normally distributed. Reference interval studies mostly depict nonparametric tests, wherein population data frequently depict skewness [1]. A precaution entails that an inappropriate analysis regarding sound data can direct the practitioner toward drawing a wrong conclusion.

5. Reference interval studies

Lab examination data are deployed in making clinical diagnoses, managing therapy, and assessment of physiologic functionalities. Interpretation of lab data implies that the clinicians are comparing the evaluated test outcome from a certain patient with a certain reference interval. Nevertheless, reference intervals encompass all the data values defining the observations' range. All normal ranges are indeed referenced intervals, but not all reference intervals outstand to be normal ranges. The following example asserts the validity of this statement.

Considering the reference interval that accrues to therapeutic drug levels, a "normal" individual will not have any drug dominating his/her system, while a patient undergoing therapy exhibits a certain target range. The theory of developing reference intervals involves standardization of collection approaches, application of statistical techniques in analyzing reference values, and selection regarding reference populations. There are two key forms of reference interval examinations—verification of reference interval and establishment of reference intervals. Establishment of a reference interval prevails once there lacks an existing analyte or rather methodology regarding the reference or clinical lab entitled to hold the comparative studies. This approach is labor intensive besides being costly since it entails lab resources at nearly all levels and may call for 120–700 study persons. Nonetheless, verification of a reference interval, or rather transference, is done with an aim of confirming the validity accruing to a prevalent reference interval provided that the analyte is utilizing identical analytic systems (methodology and/or instrumentation). This approach is fairly common regarding the operation of the clinical labs and can call for a few study individuals like 20. In addition to that, application of reference interval can be categorized into three primary classes—diagnosis of a condition or disease, monitoring a physiologic condition, and therapeutic management. The paradigm for verification or establishment of reference intervals can be damn overwhelming notably for the clinical lab that deals with multiple degrees of reference intervals-partitions. The personnel, resource, and cost requirements necessitate that the underlying reference interval examination ought to be well structured and defined to yield timely and accurate reference intervals for the productive clinical application.

5.1. Selection of reference interval study persons

This identification of people worth of inclusion in a certain reference interval experiment necessitates definition of detailed exclusion/inclusion criteria. The inclusion criteria state the factors crucial for use in the study, whereas the exclusion criteria specify the factors that make persons inappropriate for the experiment. Selection of the right individuals facilitates the acquisition of optimal specimens that exhibit acceptable degrees of confidence. Moreover, collecting the appropriate information regarding the exclusion and inclusion criteria, like donor health status, frequently necessitates a well-documented and confidential questionnaire as well as a consent form. An additional consideration regarding the selection of the individuals encompasses additional determinants that may necessitate partitioning persons into subgroups. Such subgroups may need separate reference interval experiments.

5.2. Pre-analytic and analytic considerations

After selection of individuals for a specific reference interval examination, a key consideration entails the pre-analytic and analytic variables capable of influencing certain lab tests. Control and standardization of both variables are crucial for the generation of valid reference intervals. Additionally, some approaches are damn sensitive to interferences. For instance, mass spectrometry is resistant to interferences, while chemical approaches are sometimes highly sensitive to the same. Additional consideration entails the specific reagents used since altering to a modern agent amidst a reference examination can widen the underlying reference interval or rather transform the data distribution, maybe from bimodal to normal. Universally, a valid

reference interval study necessitates extensive knowledge regarding the analyte, methodology, instrumentation, and analytic parameters.

Furthermore, plotting a reference approach versus a test approach and establishing a linear regression are key for determining whether to verify or establish a new reference interval. A correlation coefficient of one, the slope of one, and y-intercept of zero assert that the two approaches concur and hence a mere reference interval verification examination is necessary. Conversely, a considerable difference between the two approaches implies the necessity for establishing a modern reference interval. Nonetheless, analysis of reference values involves four key approaches—bias, confidence interval, parametric method, and nonparametric approach. The nonparametric approach is suitable for the majority of the reference range intervals involving analytes that are not normally distributed. A parametric approach is valid for the observed values that depict a Gaussian distribution. Confidence interval involves a range of values covering a specific probability and it serves to show the estimates' variability besides quantifying the variability. Bias implies the difference between the reference mean and the observed means wherein a negative bias implies that the reference value exceeds the test values, whereas a positive bias implies that the test values are higher [5]. Nonetheless, there is a current development regarding statistical software packages like MedCalc, JMP, SAS/STAT, Minitab, EP Evaluator, and GraphPad Prism [1]. This development has made a manual determination of reference intervals rare.

5.3. The statistical evaluation of reference values

It consists of [6]:

i. Segregation of the reference values into suitable groups

ii. Assessment of the dispersal of each group

iii. Finding out the outliers

iv. Establishment of the reference limits

5.3.1. Segregation of the reference values into suitable groups

The corresponding reference values and the reference individuals should be segregated into suitable groups according to age, sex, etc. It is done with the purpose of reducing biological "noise" and variations among the people. Various authors have developed various criteria for segregation and statistical methods for this purpose [7].

5.3.2. Assessment of the dispersal of each group

Graphical representation of the dispersal of each group should be done, and the data should then be assessed.

5.3.3. Finding out the outliers

An outlier means a person or thing situated away or detached from the main body or system or a person or thing differing from all other members of a particular group or set. In Ref. value setup, it means a value which is incorrect or inaccurate that drifts or digresses from the

established or accepted reference values. Too many methodical problems arise during the determination of the outliers; some methods developed in 2005 seems to be the solution for it [8].

5.3.4. Establishment of the reference limits

Many parametric and nonparametric methods are available for this [6–9].

6. Diagnostic efficiency

Universally, healthy patients depict entirely different lab values from the patients having epidemics. Nonetheless, lab values typically overlap, especially between various populations. Diagnostic efficiency is the key determinant regarding the appropriateness of a test at detecting and foretelling the prevalence of a disease. Diagnostic efficiency can encompass predictive values, specificity, and sensitivity. Diagnostic sensitivity entails the potential of a test regarding detection of a certain condition, whereas diagnostic specificity involves a test's potential to correctly detect the absence accruing to a certain condition or disease [10]. A positive predictive value depicts the probability of a person having a certain disease or condition once the test is not normal, whereas negative predictive value depicts a chance for an individual not having a certain condition or disease once the test is in the reference interval. The measures of diagnostic efficiency quantify the usefulness of a test regarding a certain condition or disease. Analytical sensitivity entails the lower extent of detection regarding a certain analyte, while clinical sensitivity encompasses proportion of people who test positive to show the presence of the underlying disease. True positives (TPs) are the patients confirmed by the test to have a certain disease, while those classified as not having the condition are false negatives (FNs). Contrary to specificity and sensitivity, predictive values rely on the condition's prevalence in the population under study. Measures of the diagnostic efficiency entirely rely on the distribution accruing to test outcomes for the TPs and FNs and the cutoff utilized in defining abnormal extents. Definition of effective cutoff necessitates laboratorians to frequently deploy a graphical tool—the ROC (receiver operator characteristic) [11].

7. Method evaluation

The value accruing to medical lab service depends on its potential to offer accurate and reliable test outcomes. Method evaluation targets at the production of outcomes within clinically acceptable error to assist physicians to optimally merit their patients. Regarding the regulatory issues of method evaluation, the Centers for Medicare and Medicaid Services (CMS) and the FDA outstand as the key government agencies influencing lab testing approaches in the USA. Invariably, the FDA controls lab reagents and instruments, while the CMS controls the Clinical Lab Improvement Amendments (CLIA) [12]. Nevertheless, method selection entails gathering the technical information linked to the test, its scientific literature, and presentations. Key reasons for selecting a new approach to entail a reduction of costs, improving efficiency and quality of outcomes besides amplifying client satisfaction. A method pre-evaluation follows

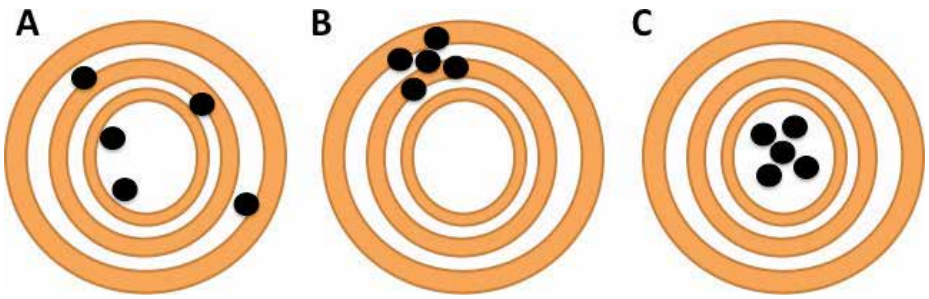

Figure 2. Graphic representation of (A) imprecision and inaccurate, (B) accurate but imprecision, and (C) precise and accurate [1].

which involves analysis of several standards with an aim of verifying the replicate analysis and linear range of two controls in order to acquire estimates regarding short-term imprecision. Inaccuracy and imprecision should be compared to the highest allowable error linked to medical criteria wherein acceptability prevails when the estimates are below the allowable highest error. After determination of imprecision, accuracy can be estimated via recovery, interference, and the patient-sample comparison. The key aspect regarding method evaluation entails determining whether the total error (systematic and random errors) does not exceed the allowable analytic error [13, 14]. The CLIA publishes the allowable analytic errors by the federally mandated proficiency examination (**Figure 2**).

8. Quality control

QC entails the systematic tracking of the analytic procedures in the lab to detect the analytic errors that prevail during analysis and finally curb reporting of incorrect test outcomes. An analytic approach is functioning optimally if the expected values lie within the underlying control limits. QC materials entail the specimens that are analyzed for QC functionality, and they ought to be of the similarity matrix as the tested specimens. Additionally, QC charts graphically denote the control material's observed values over time within the control limits. Multi-rule simplifies the various control rules to judge if an analytic approach is within the control or not. Proficiency testing is key to validating key measurement processes.

9. Quality management

Regarding quality improvement, Lean Six Sigma offers an infrastructure and methodology for quality enhancement. Additionally, define, measure, analyze, improve, and control (DMAIC) approach facilitates quality promotion. Regarding metrics, Lean Six Sigma targets at reducing cycle time, whereas Six Sigma targets at reducing error. Combining both ideologies yields a synergetic positive influence on the quality and process performance [15].

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Chapter 3

Quality Management Systems for Laboratories and External Quality Assurance Programs

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Additional information is available at the end of the chapter

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Abstract

A quality management system (QMS) plans, controls, and improves the elements that impact on the achievement of the desired results by the laboratory and on the satisfaction of the users. There are different standards that establish requirements for the implementation of a quality management system for laboratories, and a cross comparison between them is shown. Additionally, external quality assurance or assessment (EQA) programs offer multiple benefits to laboratories: method validation, comparing of results with other laboratories, testing problem identification, accreditation requirement compliance, and credibility. In order to control the quality of the procedures, these programs are a tool to keep the laboratory procedures and every variable involved in (staff, equipment, and method) well controlled. In the frame of a quality management system, benefits from external quality assurance programs are discussed, and different available designs are reviewed. On the other hand, previous benefits will be real only if reported results for each program are analyzed in detail. Because additional advantages are achieved when the EQA results are integrated in the quality management system of the laboratory, a procedure is proposed. In addition, results from external quality assurance programs corroborate the usefulness of internal controls implemented by the laboratory as part of its quality management system.

Keywords: quality management systems, external quality assurance, quality control, laboratories, harmonization, quality indicators

1. Introduction

A quality management system (QMS) is formed by a series of coordinated activities that are carried out on a set of elements to achieve the quality of the products or services offered to the customer or user. In the case of a laboratory, the accuracy, reliability, and timeliness of the analytical results reported define its quality, and all aspects of analytical operations should be controlled [1].

The QMS plans, controls, shares, and improves the elements that influence the fulfillment of user requirements and satisfaction as well [2].

An alternative definition of a QMS is through the meaning of each word separately, according to the ISO 9000:2015 quality management system—fundamentals and vocabulary:

- System: a set of interrelated or interacting elements.
- Management: coordinated activities to direct and control an organization.
- Quality: degree in which a set of inherent characteristics of an object (product, service, process, person, resource, etc.) meet the requirements (established need or expectation, generally implicit or mandatory).

We can conclude from these three sentences that the business, planning, and control activities performed on a set of elements to achieve quality represent a QMS.

Many processes are performed in laboratories to guarantee the accuracy, reliability, and traceability of the results, avoiding that any error affects its users. All those processes make a necessary quality management system that controls, detects, and tracks them.

2. International standards for laboratories

Requirements from ISO 9001 for the quality management system implementation and certification are the most widely international standards used by laboratories.

ISO 9000 documents provide guidelines for manufacturing and service industry quality and can be applied to many kinds of organizations. ISO 9001 addresses the general requirements for integration of a quality management system [1] in companies' activities from different productive sectors, including laboratories independently of its size and preserving the organization characteristics. ISO 9001 is characterized by a process-based approach, hence establishing common processes to any activity or organization, product development, or service delivery (e.g., documentation control, equipment maintenance, traceability, or staff training). Specifically, ISO 9001:2015 indicates the issues whose records must be kept:

- Quality management system and its processes
- Quality objectives
- Monitoring and measurement of resources
- Competition
- Monitoring, measurement, analysis, and evaluation
- Internal audit
- Review of management
- Nonconformity and corrective actions

In addition, there are two ISO standards especially focused on laboratory accreditation that will be detailed below:

- ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories (Geneva: International Organization for Standardization)
- ISO 15189:2012. Medical laboratories—requirements for quality and competence (Geneva: International Organization for Standardization)

Accreditation is an additional level in quality than certification. Anyway, ISO standards are voluntary norms at an international level and were created in order to standardize different activities to achieve high-quality products and services. However, accreditation is already a requirement in different government agencies for laboratory registration. Therefore, voluntary norms can become enforced in some countries and in some productive sectors.

Other important international standards for laboratories have been developed and provided by the Clinical and Laboratory Standards Institute (CLSI) by means of a consensus process from many stakeholders including the global laboratory community. These CLSI consensus-based medical laboratory standards are addressed to continually improve the testing quality, safety, and efficacy promoting medical care excellence. The quality management system model generated by CLSI is based on 12 key elements and is fully compatible with ISO standards for laboratories [1]. The CLSI has published two prominent reference documents for the clinical laboratory:

- A Quality Management System Model for Health Care (Document HS1-A2)
- GP26-A4 Application of a Quality Management System Model for Laboratory Services (fourth edition)

GP26-A4 is easy to implement because of its alignment with a variety of laboratory and accreditation standards, which helps requirement compliance in laboratories [3].

Good laboratory practices (GLPs) represent a quality management system related with organizational processes and normalized conditions, under which nonclinical health and environmental safety studies are planned, performed, controlled, recorded, archived, and informed. The main objectives of GLPs are:

- Resources optimization
- People, environment, and experimentation animals' protection
- Establishment of standardized operating methods
- To guarantee the quality and reproducibility of study results

However, GLPs is not focused on the continuous improvement.

On the other hand, good manufacturing practices (GMPs) specifically control the production variables that affect the final quality of medications according to the quality standards appropriate to the intended use [4].

Finally, there are many other standards for laboratories that are conducted only to specific laboratory areas, analysis, or programs and zones such as the standards developed by the World Health Organization (WHO), and some countries have even provided national quality standards for laboratories that are not the scope of this review.

3. ISO standards applicable to laboratories

As it was mentioned before, unlike ISO 9001 certification, the following standards accredit and are more often used by laboratories that wish or need to prove their proficiency.

3.1. ISO/IEC 17025: competence of testing and calibration laboratories

ISO 17025 establishes a set of requirements that must be met by entities performing tests and/or calibrations, including sampling. This standard is used by laboratories that want to develop and implement a quality management system for their services and to achieve laboratory accreditation. It establishes a model for the evaluation of the technical competence of the laboratory through a third-party audit.

ISO 17025 applies to all laboratories, regardless of the number of employees or the extent of the scope of testing or calibration activities and either for other organizations or individuals or their own organization. It covers tests based on standardized, non-standardized, or laboratory-developed methods.

ISO 17025 is formed by two groups of requirements:

- Management requirements: very similar to ISO 9001, they are related with the quality management of the laboratory.
- Technical requirements: aspects that influence directly on the results of laboratory testing and calibration activities.

Benefits of operating within a QMS like this are recognized by analysts thanks to the revenue increasing of laboratory business [5].

3.2. ISO 15189: competence of clinical laboratories

ISO 15189 includes all the requirements that medical laboratories in charge of human biological sample analysis must comply to guarantee that:

- They have a quality management system.
- They are technically competent.
- They have the capacity to produce technically valid results.

In the same way that ISO 17025, this standard does not certify but accredits specific testing techniques in function of the laboratory needs. Achieving ISO 15189, clinical laboratories

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demonstrate in an objective way and accredit that they have the necessary quality and technical competence, with a correct functioning of the laboratory. In an ISO 15189 laboratory, their processes are controlled and satisfy the technical requirements to ensure clinical diagnosis information, establishing a confidence framework between society, patients, doctors, and the laboratory service [6]. This standard is a good option for high-quality clinical laboratories and services [7].

Comparing ISO 9001, ISO/IEC 17025, and ISO 15189 (**Table 1**), ISO/IEC 17025 requires that "technical requirement" processes are documented. In other words, those factors that contribute to the accuracy, reliability, and validity of tests and calibrations, such as the staff, environmental conditions, equipment, or samples, must be recorded. These requirements related to human resource management (specifically in terms of qualification and competence or infrastructure (to guarantee test conditions) are due to test and calibration specificity and sensitivity.

ISO 15189 extends also its scope to analytical, pre-analytical, and post-analytical phases to establish interaction mechanisms between patients, medical staff, and the laboratory.

ISO 9001	ISO/IEC 17025	ISO 15189
Foreword	Foreword	Foreword
0 Introduction	0 Introduction	0 Introduction
1 Scope	1 Scope	1 Scope
2 Normative references	2 Normative references	2 Normative references
3 Terms and definitions	3 Terms and definitions	3 Terms and definitions
4 Context of the organizations	4 Management requirements	4 Management requirements
4.1 Understanding the organization and its context	4.1 Organization	4.1 Organization and responsibility of management
4.2 Understanding the needs and expectations of interested parties	4.2 Management system	4.2 Management system
4.3 Determining the scope of the quality management system	4.3 Document control	4.3 Document control
4.4 Quality management system and its processes	4.4 Review of requests, tenders, and contracts	4.4 Contracts for the provision of services
	4.5 Subcontracting of tests and calibrations	4.5 Analyses carried out by subcontractor laboratories
	4.6 Purchasing services and supplies	4.6 External services and supplies
	4.7 Service to the customer	4.7 Advisory services
	4.8 Complaints	4.8 Resolution of claims
	4.9 Control of nonconforming testing and/or calibration work	4.9 Identification and control of nonconformities
	4.10 Improvement	4.10 Corrective action

ISO 9001	ISO/IEC 17025	ISO 15189
	4.11 Corrective action	4.11 Prevention action
	4.12 Prevention action	4.12 continuous improvement
	4.13 Control of records	4.13 Control of records
	4.14 Internal audits	4.14 Evaluation and audits
	4.15 Management reviews	4.15 Management reviews
5 Leadership	5 Technical requirements	5 Technical requirements
5.1 Leadership and commitment	5.1 General	5.1 Personnel
5.2 Policy	5.2 Personnel	5.2 Accommodation and environmental conditions
5.3 Organizational roles, responsibilities, and authorities	5.3 Accommodation and environmental conditions	5.3 Laboratory equipment, reagents, and consumables
	5.4 Test and calibration methods and method validation	5.4 Pre-analytical processes
	5.5 Equipment	5.5 Analytical processes
	5.6 Measurement traceability	5.6 Assurance of the quality of the analysis results
	5.7 Sampling	5.7 Post-analytical processes
	5.8 Handling of test and calibration items	5.8 Notification of results
	5.9 Assuring the quality of test and calibration results	5.9 Comunicación de los resultados
	5.10 Reporting the results	5.10 Management of laboratory information
6 Planning		
6.1 Actions to address risks and opportunities		
6.2 Quality objectives and planning to achieve them		
6.3 Planning of changes		
7 Support		
7.1 Resources		
7.2 Competence		
7.3 Awareness		
7.4 Communication		
7.5 Documented information		
8 Operation		
8.1 Operational planning and control		
8.2 Requirements for products and services		

ISO 9001	ISO/IEC 17025	ISO 15189
8.3 Design and development of products and services		
8.4 Control of externally provided processes, products, and services		
8.5 Production and service provision		
8.6 Release of products and service		
8.7 Control of nonconforming outputs		
9 Performance evaluation		
9.1 Monitoring, measurement, analysis, and evaluation		
9.2 Internal audit		
9.3 Management review		
10 Improvement		
10.1 General		
10.2 Nonconformity and corrective action		
10.3 Continual improvement		
Annex A Clarification of new structure, terminology, and concepts	Annex A Nominal cross-references to ISO 9001:2000	Annex A Correlation with ISO 9001:2008 and ISO/IEC 17025:2005
Annex B Other international standards on quality management and quality management systems developed by ISO/TC 176	Annex B Guidelines for establishing applications for specific fields	Annex B Comparison of ISO 15189:2007 and ISO 15189:2012
Bibliography	Bibliography	Bibliography

Table 1. Structure and items from the ISO 9001, ISO/IEC 17025, and ISO 15189 standards.

On the other hand, the choice between certification (ISO 9001) and accreditation (ISO 17025 standard applicable to testing or calibration laboratories or ISO 15189 when it is a clinical laboratory) will depend on the requirements from current or potential customers, regulatory boards, or the expected growth and development of the laboratory [8]. **Figure 1** shows the similarities and differences between certification and accreditation.

When customers need international recognition of their results or the laboratory wishes to incorporate users with international requirements, corresponding laboratory accreditation for the required tests is the best option, since it allows establishing the validity of their tests. If customers must ensure the sample traceability from the collection to result delivery, the easiest and cheapest option of quality management system is ISO 9001. At the local level, the ISO 9001 certification may be enough to provide confidence quality in the products or services offered, to be able to differentiate themselves from the competition and gain market share and public tenders, among other objectives.

Alternatively, laboratory mission, vision, and policy can include issues related with market positioning, so that specific objectives should be defined regarding to certification and accreditation in each case.

Benefits from implementation of ISO 9001, ISO/IEC 17025, and ISO 15189 in the laboratory are shown in **Table 2** [6].

Figure 1. Similarities and differences between certification and accreditation.

Benefits	ISO 9001	ISO/IEC 17025	ISO 15189
Improvement of the company image	X	X	X
Allow to gain market share	X	X	X
Improvement of business efficiency	X	X	X
Improvement of qualification to access tenders	X	X	X
Improvement of internal processes	X	X	X
Achievement of strategic objectives	X	X	X
Establishment of mechanisms for the continuous improvement of service quality	X	X	X
Achievement of customer satisfaction	X	X	X
Customer loyalty	X	X	X
Allow formal recognition of technical competence		X	X
International recognition of trials		X	X
Commitment of staff in meeting customer requirements		X	X
Development of staff competencies		X	X
Fulfilled requirement for the registration of the laboratory in governmental organisms		X	

Table 2. Benefits from implementation of ISO 9001, ISO/IEC 17025, and ISO 15189 in the laboratory adapted from [6].

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4. External quality assurance programs

"External quality assurance or assessment" (EQA) programs are a tool designed by different providers (usually medical or scientific societies) with an educational, training, and helping purpose. They allow the evaluation of the analytical performance for every variable involved (staff, equipment, reagents, and method) in comparison with the expected results. Similarly, EQA schemes are an educational tool to evaluate the competence of the laboratory in relation with specific variables. In addition to internal quality control (IQC), EQA is complementary in the quality management system. Alternatively, proficiency testing (PT) is used as external quality assurance with a regulatory purpose for laboratory licensing and/or accreditation [9].

EQA programs allow comparing the laboratories' results and informing on global variation with the objective of working toward the harmonization. This goal is extremely important because medical decisions are based on comparisons of analytical results with time or a reference interval [10].

In this sense, international societies recognize the importance of EQA provision [11]. The World Health Organization has an available manual for organizing a national EQA program for health laboratories and other testing sites, providing guidance on the international standards ISO 17043:2010 Conformity assessment—general requirements for proficiency testing and ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison. Contrary to expectations, not enough evidences of quality improvement of the analytical performance as a result of EQA participation have been reported [12].

The EQA participation process is summarized in **Figure 2**. Samples prepared by the EQA provider are sent to the laboratories for their analysis. These samples of unknown nature are handled by the laboratory from their reception until the report emission as usual samples, although trying to participate each analyzer in the whole program [9]. EQA provider receives the analytical results from all of the laboratories and prepares a confidential report with the identified deviation regarding to an assigned value [13]. Optionally, report may establish acceptance limits for the assigned value in accordance with analytical performance specifications [14] and inform about the performance evaluation of the several methods employed by participants.

Acceptance limits have been classified as [13]:

- Regulatory: for identification of laboratories with a poor performance of the analysis.
- Statistical: an acceptable result is defined by its similarity with others derived from the same method. The disadvantage of this kind of acceptance limit is that it varies between methods.
- Clinical: based on medical decisions.

In case of nonregulatory EQA participation, the laboratories should decide the proper limits for the proposed objective. When the acceptance limit is defined as the "fitness for purpose", such purpose must be specified based on external requirements [15].

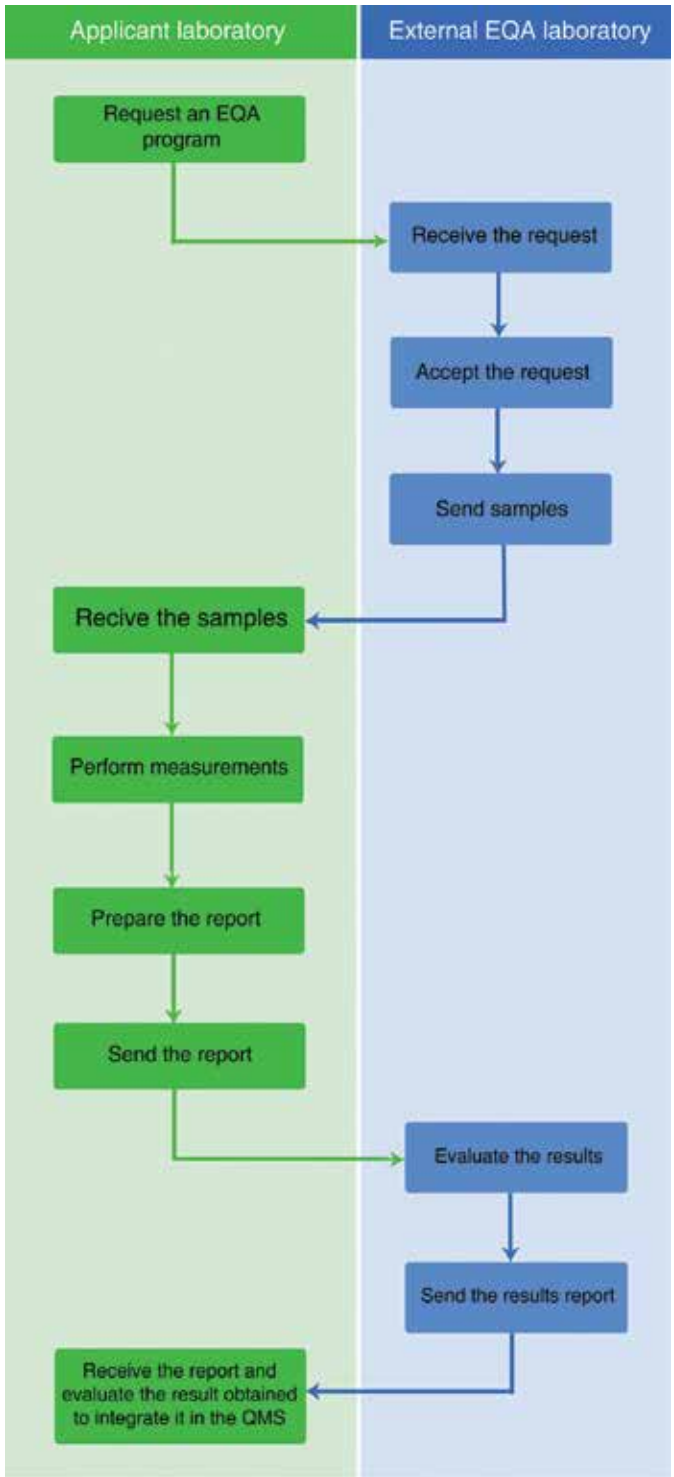

Figure 2. EQA participation process.

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The optimal EQA participation frequency has not already established, but targeted high-quality schemes with a proper number of samples are preferred instead of many schemes with a risky participation rate.

Quality of EQA programs depends on the properties of their design [16]. The use of validated commutable samples and the assigned value definition based on a reference measurement procedure or by comparison with a certified reference material makes an EQA program prominent.

An EQA sample is commutable when the result after the analysis by a variety of methods is equivalent to the result obtained from patient samples with the same amount of analytes. In other words, the results for different methods are comparable because there are no matrix-related biases in commutable samples [17, 18]. However, commutability is not always possible since enough volume of EQA samples with relevant concentrations must be prepared in homogeneous and stable conditions.

Additionally, to use biological samples as reference material is necessary in their certification precise information about their characteristics (processing, purity, characterization, "fitness for purpose", homogeneity, stability) and about their original clinical, biological, and pathological diagnosis. Only in this way, application of ISO Guide 34 requirements for reference material production to EQA samples would be achieved [19].

If commutable samples are not available, it is not possible to evaluate method accuracy. In this sense, laboratories are evaluated and classified by groups of participants with the same method and expected matrix-related bias (peer groups) because comparison to the same assigned value is impracticable. The assigned value is the group mean or median after outliers' removal or by using robust statistical tools and deviation is calculated. It is worth mentioning that the uncertainty of the estimated assigned value would be larger in a small peer group than in a bigger one [13]. Another disadvantage is that peer group evaluation is made impossible to identify a poor performance result when all reagents from the participants are affected. This is the reason why reagents' batch number should be recorded and took into consideration during evaluation by the EQA provider, contacting to the manufacturer when batch effects are observed [20, 21]. In spite of previous limitations, this type of EQA allows to measure the quality of the results with respect to the method and the other laboratories in the same group.

Independent of sample commutability, previous analysis tools are not valid for semiquantitative measures or measures reported on a discontinuous scale or where dichotomous results are provided for a continuous parameter [15].

Ideally, and with the previously commented objective of laboratories' result harmonization, international EQA programs are recommended. However, they are a nonviable option for routine use because of their cost and complexity, being precisely the challenge for EQA providers to find new solutions and overcome limitations related with EQA design [16]. In the meantime, an alternative option that has been proposed [10] would be to organize a global EQA characterized by its remarkable design (validated commutable samples and assigned value by references) for a few representative laboratories from different countries. In a second

phase, these laboratories would participate in smaller national or regional programs with an optimal design as reference laboratories. In the frame of this initiative, results from EQA should be reviewed by a professional international advisory board to inquire the root causes for global deviations.

A particular case of testing is the point-of-care (POC) technologies, which has the very prominent advantage of increasing the populations' access to diagnoses through introduction of a decentralized model. However, from the EQA program's point of view, POC analytical performance increases in the same manner the design difficulty: many EQA samples are necessary for multiple testing points, where nonspecialized staff is available with a poor and delayed participation [12]. To deal with this situation, connected devices to a central database for POC technologies have been developed to establish an efficient and on time EQA workflow. Sent EQA samples are distinguished thanks to specific IDs, obtaining a cheaper, fewer errors and simplified EQA approach for each step by means of direct data collection and analysis [22].

To be clear, EQA participation does not improve directly the quality, but it identifies and monitors poor performance issues. So, it is very important that a proactive participation is implemented in the laboratory [9], being recommended that an EQA manager is available.

The laboratory must choose an EQA organizer in function of the EQA designs offered and the own quality assurance or supporting needs of the laboratory. This selective process should be justified and documented. The choice is easier when proficiency testing with a regulatory purpose is imposed. EQA providers with professional committees and accredited laboratories are preferable. With this objective, EQA provider must inform about EQA programs' designs and especially about analytical performance specifications used in each case. This information will allow the comparison between different EQA programs, as harmonization of analytical performance specifications for the same analyte has not been achieved yet.

A proactive attitude by the laboratory is also necessary, even mandatory in the case of accreditation, for proper and timely EQA report revision. Reports from EQA providers are often used as a quality follow-up tool by auditors. Laboratory staff should know the laboratory's EQA analytical performance results by means of formal communications [9].

Three kinds of reports should be available [15]:

- A confidential and clear individual report for each laboratory, also for outliers, including its deviation regarding the assigned value and usually the acceptance limits. In addition, reports may contain the number and origin of the participants and their distribution of results to allow comparison between them and even the laboratory's performance history.
- Summary reports at the end of each scheme or program with global and anonymized information about analytical performance variation for different analyses.
- Periodic reports can be published as well to highlight the most significant results found.

A very important supporting element for the evaluation in the reports, and required by international standards, is graphical representations. Graphs are also powerful tools to show combined information from a variety of analysis with different samples, time points, or other relevant variables.

Quality improvement of the laboratory after EQA participation will be only possible if changes in the deviated processes are developed. As part of their educational, training, and helping responsibilities, EQA providers should support and collaborate with the laboratory in this phase.

Proposed corrective actions must be documented and include the steps taken to find the cause of the deviation and to solve its consequences. As a troubleshooting tool for EQA concerning analytical performance, the Norwegian Clinical Chemistry EQA Program (NKK) has developed a flowchart with additional comments in collaboration with the External quality Control of diagnostic Assays and Tests (ECAT) Foundation [13]. It is a public instrument, only valid for quantitative analysis, which proposes actions to be initiated in the format of corrective and preventive action (CAPA) documentation or root cause analysis (RCA) after deviation identification by EQA. Four points are considered in the flowchart and associated comments: the potential cause of deviation, the corresponding responsibility for this cause, a brief, and, finally, a detailed explanation about the proposed actions.

The previous points are classified according to the consecutive steps in the EQA participation process:

- Transcription errors: the most frequent cause.
- Pre-survey issues: unrelated to the laboratory. Unfortunately, sample reanalysis is necessary.
- Sample receipt or handling: derived from incorrect address information, misunderstanding of EQA provider instructions, bad integrity of the EQA sample, or lack of records.
- Test performance: new or old causes that made necessary to identify who, when, and how, to look at the internal quality control data (IQC), and to look for systematic deviations from different participations over time.
- Data handling by EQA provider: these errors are due to the statistical procedure, their identification by the laboratory being difficult.
- Report and interpretation.

To sum up (**Figure 3**), the procedure to integrate the EQA results in the QMS of the laboratory is [16]:

1. Report interpretation
2. Initiating documented corrective and preventive (whenever possible) actions
    a. To collect information about who, when, and how in relation with EQA participation, IQC data, and previous and global EQA results
    b. To find the cause of deviation
3. Monitoring of actions taken
4. New analysis of a stored aliquot left of the EQA sample
5. Revision of EQA program selection

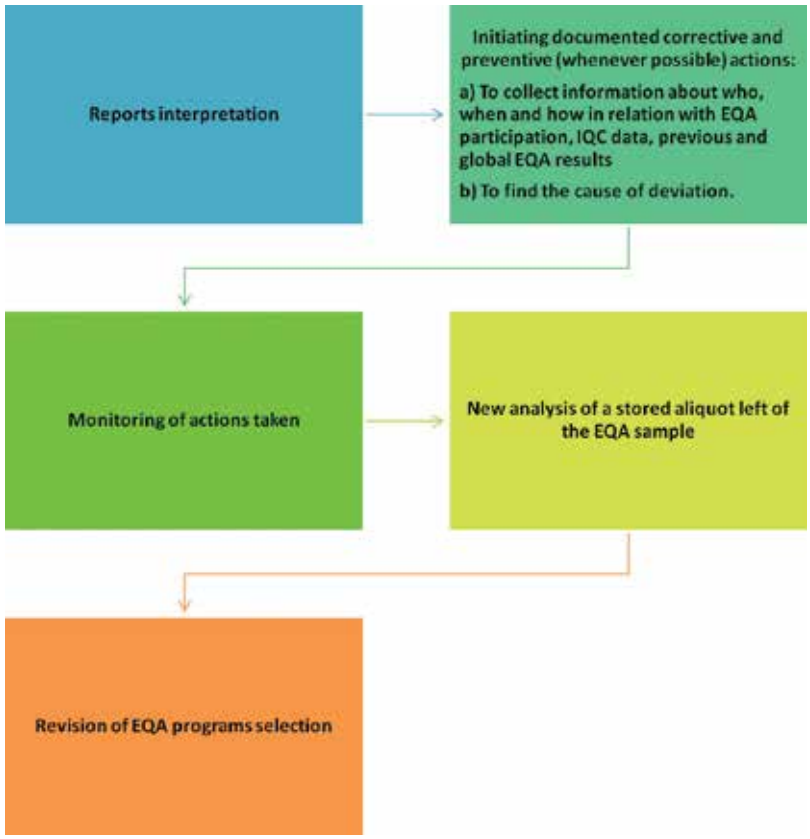

Figure 3. Procedure to integrate the EQA results in the QMS of the laboratory.

Although EQA has been usually applied to analytical performance, the EQA process should meet in the same manner the pre-analytical phases. Several efforts have been conducted trying to cover them, with three types of pre-analytical EQA schemes being categorized [23]:

- Type I: registration of procedures by means of questionnaires. Few resources are necessary to organize and participate, and relevant recommendations may be included.
- Type II: sample analysis with simulated problems. However, only limited pre-analytical deviations can be generated.
- Type III: registration of incidences. This kind of pre-analytical EQA schemes offers the opportunity to EQA providers for harmonization of quality indicators (QIs).

Pre-analytical EQA schemes are more difficult to standardize, but it is worth progressing in this sense because these phases are more prone to errors.

Furthermore, the requesting and reporting diagnostic phases should also be covered by EQA programs due to two main reasons: high rate of errors associated and the definition of quality

management system (QMS) mentioned at the beginning of this chapter of fulfillment user requirements and satisfaction [24]. The design of such programs should be developed carefully to obtain useful information.

Clinical laboratories' activities are based on evidences derived from research [10]. Biobanks are singular laboratories that provide samples for research. Differences between biobanks in preanalytical and processing methods for the same kind of samples may impact research results [25]. Therefore, EQA process provides an opportunity for harmonization in the biobanking field as well. With a main educational purpose, the International Society for Biological and Environmental Repositories (ISBER) have developed an EQA program focused on sample processing and testing [26] that represents a very important part of a biobank quality management system.

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Chapter 4

Implementation of Quality Control

Irfan Ahmad

Additional information is available at the end of the chapter

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Abstract

Quality control is a procedure for examining the problems, work processes as well as making improvements preceding to transport of products. In the global markets, the rising competition between manufacturers and producers has guided firms in recognizing the competitive benefits. However, in the previous eras, the importance has been attentive on the continuous upgrading of quality for the success of several kinds of business in present and future. Employing a universal quality assurance background will help in more effective organization and improve the quality of statistical output in international organizations. Quality values are an essential part of the quality system. They are deliberated to support regulatory requirements of the laboratories, including monitor laboratory functions and local health regulations, thus confirming the safety of the local health regulations and reliability of performance. This chapter highlights the elements essential for effective implementation of quality control.

Keywords: quality control, implementation, essential elements, quality management, improvement, six sigma

1. Introduction

Quality is a worldwide value and now has converted a universal concern. The burden of globalization has built manufacturing organizations affecting to three main competitive areas such as cost, responsiveness and quality. For the better survival, it is necessary to offer clienteles with good quality stuff, so necessary for manufacturing organizations to ensure that their procedures are constantly supervised and quality of the product is enhanced. The manufacturing company applies several techniques for quality control (QC) to increase the quality of the progression by decreasing its variability [1].

A variety of methods exist to control the products or process quality. Seven statistical process control (SPC) tools are included such as plan, do, check, act (PDCA), quality function deployment (QFD), acceptance sampling, failure mode and effects analysis (FMEA), a design of experiments (DoE) and six sigma [2]. This chapter emphasizes the implementation of quality control in companies and categorizes the factors applying for quality control techniques, the techniques used in the implementation. The inspiring factors for the companies to relate quality control and tasks challenge by companies in implementing the quality control [3–5].

Laboratory facilities are an important constituent of quality health care. It can be employed efficiently at each level of health care system, involving point-of-care and primary health care testing. Results of the quality laboratory are requisite to support justify, monitor, treatment and clinical diagnosis, for the purposes of epidemiology, surveillance and control of disease at public health importance, and to deliver the initial warning of disease epidemics. This increases the accuracy of health evidence and endorses the national health planning effectively. The aim of establishing laboratory quality standards is to confirm the confidence of patients, increase the accuracy of test results, communities and clinicians in the importance of laboratory testing, and to update patient management [6]. Entirely laboratory activities might be subject to errors, and many studies have exposed that errors in the laboratory can appear in all the phases of diagnostic processes. The examples of errors that may occur in all phase are given below.

1.1. Pre-analytical phase

- Incorrect test selection or test request
- Incomplete request forms of laboratory
- Incorrect collection of specimen, inadequate quantity, improper labeling and transportation

1.2. Analytical phase

- Use of defective equipment, inappropriate use of an equipment
- Use of expired or substandard reagents
- Incorrect storage and reagent preparation
- Incorrect procedures; non-adherence to internal quality control (IQC) or standard operating procedures (SOPs)

1.3. Post-analytical phase

- Inappropriate reporting or recording
- Incorrect calculations, transcription or computation
- Send the results too late to the clinician
- Inappropriate interpretation of the results

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2. Quality control

Quality can be described as achieving customer requirement or specification, without any deficiency. A product is considered to be great in quality if it is working as reliable and expected. Quality control denotes to activities to confirm that produced articles are achieving the highest promising quality. Furthermost of tools and techniques to control the quality are statistical procedures. The techniques for the quality control can be categorized into the basic, intermediate and advanced level, although there is no unanimity amongst researchers about it. For example, Xie et al. [7] deliberate the DoE as an intermediary level technique while Antony et al. [8] classified that technique as advanced. Nonetheless, the contents are more significant rather than the classification. Amongst the basic techniques, SPC is a statistical method for supporting the supervisors, operators and managers to accomplish quality and to remove special causes of inconsistency in the process [9]. The early role of SPC is to stop rather than process deterioration or recognize the product, but Xie et al. [7] propose for its new role to vigorously identifying prospects for the improvement of the process. The foremost tools in SPC are control charts. An essential knowledge of the control charts is to analyze the hypothesis that there are few common reasons of alternative versus variability, that there are exceptional causes by continuously observing the process. The manufacturing company could avoid defect items to be administered in the subsequent stage and to take instant corrective action while the process exists to be out of control [10].

DoE and Taguchi approaches are influential tools for the development of product and process. Taguchi methods, for example, the purpose of manufacturing products or process that vigorous to non-desirable turbulences such as manufacturing and environmental variations. Though, the request of these two approaches by industries is inadequate [11]. Antony et al. [8] delineate the problems in the application including the inappropriate understanding of statistical ideas in the procedures; therefore recommend an approach for the implementation. Procedure ability study is an effective technique to examine the ability of a procedure to produce items that meet specifications.

The process gains fast raising attention because of increased utilization of the quality system (QS9000), where to take advantage of method capability study are demanded [12]. The outcome obtains from capability study may want some modification of process employing some other statistical technique, for example, DoE or SPC. Furthermore, Motorcu and Gullu [13] and Srikaeo et al. [14] conducted a capability study in which process capability production and stability machine tool were assessed and crucial procedure to diminish poor quality production was carried out employing other statistical methods.

Failure mode and effects analysis (FMEA) is a well-known technique to identify the point where precisely problems can take place as well as to urgencies feasible problems in the order of their difficulty [15]. This tool is valuable to troubleshoot problems in the process, i.e. process FMEA and to recognize problems in the product, i.e. design FMEA [7]. Additionally, six sigma is also a known statistical device for confirming the fault-free products via nonstop progress and six sigma application has been chiefly employed in manufacturing industry. However, use of six sigma in the software development is a case of the non-manufacturing

industry [16]. The term six sigma instigated by Motorola as well as various motivated international organizations have fixed goal concerning a six sigma level of implementation [17].

Moreover, acceptance sampling is alternative statistical techniques that concluded whether to take or refuse a quota based on the information from the sample. The request for the approval of sampling permits industries to minimize the product demolition through examination and to raise the inspection capacity and efficiency. The request of getting sampling has been chiefly employed in manufacturing industry; however, Slattery [18] reported its application in non-manufacturing industry.

3. Implementation of the quality control system

Quality standards are an integral part of the quality system. They are designed to help laboratories meet regulatory requirements, including local health regulations, and monitor laboratory functions, thereby ensuring laboratory safety and consistency of performance. A quality system can be developed in a step-wise manner and implementation (**Table 1**).

The methodologies for the implementation of quality control can be differ in diverse organizations. Irrespective the methodologies of the continuous improvement program, each organization desire to use the proper tools and techniques in the process of implementation. The selection of tools and techniques is depend on the demands and applied appropriately to the approach and process.

The PDCA is an essential concept for quality improvement processes, easy to understand and followed by most of the organizations. The most significant characteristic of PDCA lies in the "act" phase after the completion [19]. The six-sigma procedure is consistent and delivers a rigorous outline of results concerned with management. It must be distinguished that the greatest results from six-sigma are accomplished and eradicating unproductive procedures, especially when the members of the team are new to the concerned tools and techniques [20, 21].

3.1. Implementation of laboratory quality standards

The implementation process for laboratory quality standards must follow a stepwise attitude conferring to an implementation strategy drawn up by the national laboratory, in discussion with the National Laboratory Coordinating Committee. Certain countries can desire to progress national laboratory quality standards for all level of health care system.

Implementing laboratory quality standards guidelines are as follows.

3.1.1. National level

1. Achieve nationwide agreement for established standards through peer review.
2. Achieve consent to established standards via the suitable nationwide experts.

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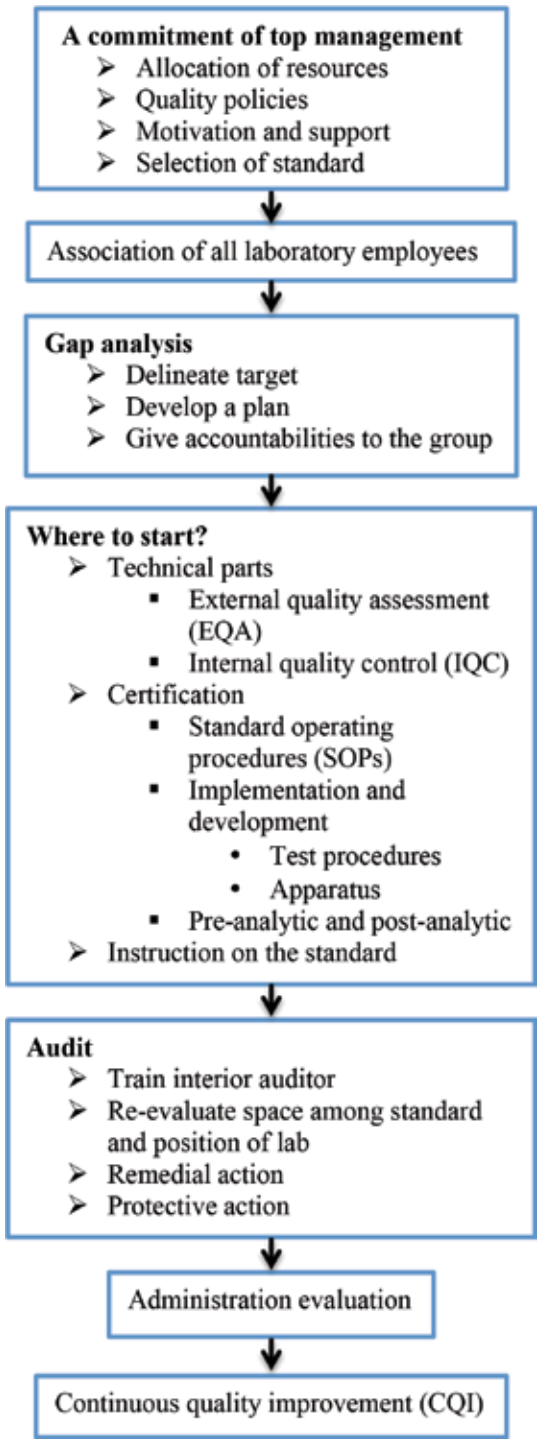

Table 1. Key steps in implementing a quality system.

3. Make a short-term, medium-term and long-term implementation plan for objectives, timelines and activities, and revealing yearly budgets.
4. Recognize suitable implementing agencies such as non-government, governmental agencies, and the private sectors.
5. Explain partaking health facilities and institutions.
6. Use existing SOPs, checklists, record forms, guidelines and appraisal forms, audit checklists, recording formats etc. or develop the documents for the country specific.
7. Establish the national procedures for the referral of samples and laboratory networking.
8. Establish the annual plans with budgets.

3.1.2. Laboratory level

A similar procedure will be mandatory by different laboratories. The head of the laboratory will require taking a leadership role and involving all the staff. Several changes are informal to implement and some are extra expensive or tougher to implement.

The changes that make the implementation of quality control simple and easy:

1. Introduction of SOPs for specific activities or procedures. This can be the collection of the sample, comprising phlebotomy for the investigation of a specific analysis.
2. Arrange meetings with the users consistently. This will inform the users of the service to upgrade the quality of laboratory.

4. Challenges and future trends in QC implementation

Quality control by manual approach could be established in several companies, such as, to observe cuprum pipe pressing procedure, specific control chart is employed to identify the existence of precise distinctions in the process. Furthermore, the chart is made by hand hence it needs a large amount of time period for chart preparation. However, the workers appear found to be more comfy with hard copy records as well as the manual process in making a record for the created items. The absence of confident in soft copy file supposed to be dread that someone may interfere and alter the data that can depreciate the company reputation.

Earlier studies have been showed comparable difficulties in applying quality control between native manufacturing institutes. Among the serious difficulties are concerning insignificant process observing, incapability to accomplish data analysis, the accomplishment of control chart just on the completed products and not in a real-time approach [22, 23]. Additionally, real-time quality control additionally affords countless competence to the management since it takes time to make manual control charts as well as the time permitted to accomplish significant data analysis, is reduced [24]. Study by Mohd Nizam et al. [25, 26] and Rosmaizura et al. [27] show obstacles in developing an online Statistical Process Control (SPC) system

and the outcome of the study illustrate that aspects associated with highest management support, inter-departmental correlation, budgets to improve the system and education on SPC are hindering manufacturing institutes via showing real-time process censoring. It is well documented that strong obligation by top management is very important for the fruitful accomplishment of SPC [28, 29].

In forthcoming days, it is supposed that manufacturers will face a progressively undefined exterior atmosphere through an increasing consequence of alterations in worldwide competition, technological improvement and customer necessities. Flexibility, cost, time and quality are considered as amongst the very significant competitive weaponry for the success of manufacturing companies. Manufacturers face the task of refining the efficacy and lowering prices. Hence, QC techniques would be constantly used to support the organizations to develop, revolutionize their goods and progression in order to be acknowledged by customers. Because of the rising concern on maintainable place and source for the upcoming generation, manufacturers are expected to give more consideration to the environmental effect from their operations. So, application of environment preservation, atmosphere friendly industrial practices and green technology seem to be dominant.

5. Conclusions

All the employees incorporate the concepts for implementation of quality control in a laboratory or organization. That will give massive benefits for the improvement of quality control. Though the program of quality assurance is still independent to monitor the process of quality control. Implementation of QC may require a change during the setup of quality management system. The encouraging features for the companies to concern quality control arise inside from the organization, parental company and/or externally from the customer. The companies use widely SPC and acceptance sampling. DOE, Taguchi methods, Six Sigma, and capability studies are missing to be used by the industries, because of the lack of knowledge in the technique. They fulfill the criteria for the laboratories requirement such as health regulation, consistency in performance, laboratory functions and safety. Three aspects influence the quality control procedure in the firms, such as the capability to quantify product specification contentment; simplicity in the use of the technique; and capability to progress acute characteristic and yield difficulty. Hence QC technique will combine all these environmental concerns like its significant elements and ease and quickness for use would be the probability for QC techniques of the future.

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Chapter 5

Systematic Error Detection in Laboratory Medicine

Amir Momeni-Boroujeni and Matthew R. Pincus

Additional information is available at the end of the chapter

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Abstract

Measurements in laboratory medicine have a degree of uncertainty; this uncertainty is often called "error" and refers to imprecisions and inaccuracies in measurement. This measurement error refers to the difference between the true value of the measured sample and the measured value. One of the types of error is systematic error, also called bias, because these errors errors are reproducible and skew the results consistently in the same direction. A common approach to identify systematic error is to use control samples with a method comparison approach. An alternative is use of statistical methods that analyze actual patient values either as an "Average of Normals" or a "Moving Patient Averages." Fundamental questions should be decided before a quality control method is used: how are weights assigned to the results? Is preference given to more recent samples or to the older samples? How sensitive should the model be? In this chapter, we will expand the fundamental notion of systematic error and explain why it is difficult to identify and measure and current statistical methods that are used to detect systematic error or bias.

Keywords: bias, systematic error, measurement uncertainty, bias detection, method comparison, patient average methods

1. Introduction

The role of clinical laboratory is to measure and test patient samples. These measurements are a central part of modern clinical management; they are used by clinicians to diagnose disease states, to guide treatment course and to determine prognosis. The modern clinical laboratory uses a plethora of instruments to quantify and measure different analytes and reports results that are used by clinicians. The most important metrics that a test must possess to be used in clinical laboratory are technical accuracy and precision [1].

A test is technically accurate if it produces valid information. A precise test will produce similar results when the test is repeated multiple times. Accuracy (or rather trueness) is a measure of the

proximity of the test results to the true value. Precision measures reliability and reproducibility. These metrics are complementary and a good clinical test needs to be both accurate and precise [2]. Some have suggested that trueness should be used to refer to the agreement of the measurement to the true value and accuracy to encompass both trueness and precision.

Accuracy and precision are related to a concept called measurement error: every measurement is associated with a degree of error or uncertainty. The goal in laboratory medicine is to minimize the measurement error so that it does not adversely affect the clinical decision-making process. Measurement error can never be truly nullified, but it can be decreased to a scale that is acceptable by clinicians, laboratory directors and laboratory regulatory agencies [2, 3].

Measurement errors can be random, i.e. they can be unpredictable. All measurements have random error. Random errors are due to unpredictable variations in sample, instrument, measurement process or analysis and it can be said to follow a Gaussian distribution, i.e. random error follows randomness and chance and thus laws of probability apply to random error. As the instruments get more precise the Gaussian distribution of the random error gets narrower and the random error decreases. At the same time, if we repeat an experiment or test multiple times we can average out random error from our measurements. i.e. the mean of multiple repeated measurements gets closer to the true value as the number of repeats increases. This forms the basis of reporting confidence intervals for measurements [2, 4].

Bias or systematic error is a form of measurement error that skews the results to one side. Repeating the measurements cannot eliminate bias. In other words, bias is a non-zero error which will consistently affect the results and can show a problem with the measurement process often requiring corrective action. The corrective action can be in form of calibration by introducing a correction factor or by changing components of measurement. Systematic error can be short-term or long-term, with very short-term systematic error often manifesting as random error.

Systematic error and random error have a cumulative effect on the measurement results (**Figure 1**). Thus, measurement error is often considered as total error with both bias and random error contributing. Laboratories often have limits for total error, bias and random

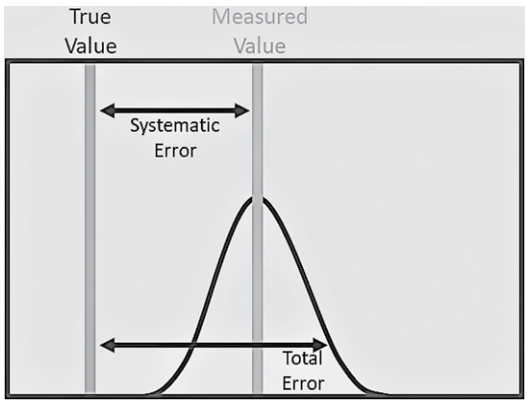

Figure 1. This figure depicts the cumulative effect of systematic error and random error. The X-axis represents the value determined and the Y-axis plots the frequency of occurrence of each value.

error. All tests need to be checked continuously for presence of error and identifying systematic error is part of the function of a clinical laboratory. The measurement error can be regarded as a noise that can obscure the signal or true test value. In the presence of noise, drawing conclusions from the signal that may change the true value in a clinically significant manner risks jeopardizing the patient's health. As a result, the lab should strive to identify noise, minimize it or reduce its impact on patient outcomes. In this regard, systematic error is especially dangerous since it will skew the test results in a manner that cannot be corrected by repeat measurements. Unfortunately, systematic error can be very difficult to identify and/or quantify. In this chapter we focus on approaches for identification of systematic error using within-laboratory comparisons [5, 6].

2. Systematic error detection using quality control experiments

Simply stated, the aim of quality control experiments is to determine the performance of the laboratory tests with measuring of known samples or references, that is, samples in which the true value of the analyte being tested is known. These methods are mainly set up to detect random error and check instrument precision. However, the same results can be used to detect bias and systematic error [7].

The laboratories can use certified reference materials to measure and identify systematic error. If the reference sample is measured with each analytical run, you would expect the results of the reference sample measurements to show a random distribution around the true value, yet if the results are consistently lower or higher than the reference value then you would suspect that a bias exists [2, 8].

For systematic error measurement, a method comparison method is needed to identify systematic error. Any systematic error found needs to be corrected using a recovery experiment and calibration.

2.1. Levey-Jennings plots

The first step in identification of systematic error is to visually inspect the quality control process. Levey-Jennings plot shows the fluctuation of reference sample measurements around the mean against time. The chart's reference lines include control limits, 2 standard deviation lines, 1 standard deviation lines and the mean reference line.

The mean, standard deviation and the control limits are calculated by a replication study where the certified reference material is repeatedly measured. The repeated measurements allow for calculation of mean and standard deviation of the control sample levels. The trial limits are mean ± 3 standard deviations. The next step is to eliminate the replication study results that are beyond the 3 standard deviations. Then the mean and standard deviation are recalculated and the trial limits are again set. Again, results beyond the trial limits are excluded. The process continues until all the remaining results are within the trial limits. These final trial limits, mean and standard deviation are set as the reference measures for that reference sample.

The number of replication studies to perform can be calculated based on the number of acceptable failures. The sample size calculation is based on set levels of confidence and reliability. Confidence (accuracy) is the difference between 1 and type I error rate. Reliability is the degree of precision. For a failure rate of 0 (i.e. we are not allowing any incorrect results), the equation can be stated as:

$$n = \frac{\ln(1 - confidence)}{\ln(reliability)} \tag{1}$$

The confidence level is often set at 0.95 and reliability at 0.90 or 0.80. If we allow failure events, then the calculation of the sample size is based on the following equation:

$$1 - Confidence = \sum_{i=1}^{f} \binom{n}{i} (1 - Reliability)^i Reliability^{n-i} \tag{2}$$

where f is the failure rate and n is the sample size.

In a Levey-Jennings plot the X-axis represents time and Y-axis represents the measured value. Reference lines are drawn parallel to the X-axis corresponding to mean, mean ±1 standard deviations, mean ±2 standard deviations, and mean ±3 standard deviations. The next step is to plot measured values of the reference material for each run on the plot (**Figure 2**).

2.2. Westgard rules

Westgard rules are a set of guidelines set by Dr. James Westgard for identification of random and systematic error in laboratory quality control experiments. They are based on repeated measurements of at least two reference samples with each analytical run. Some of the Westgard rules are

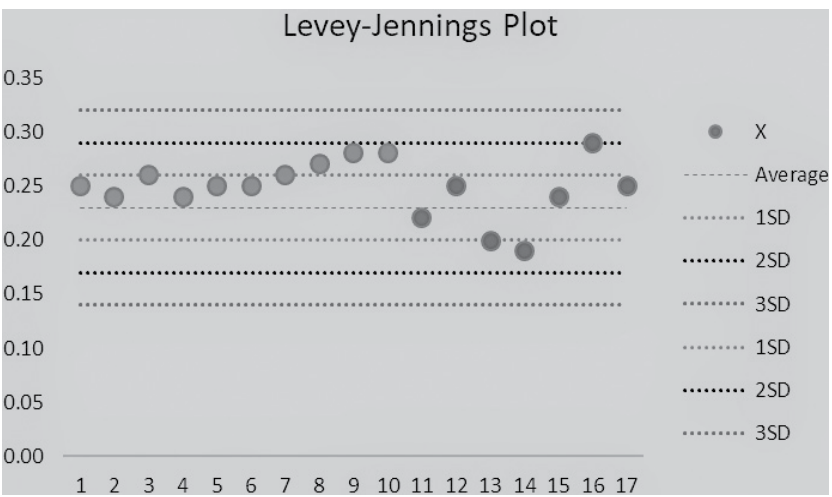

Figure 2. An example of a Levey-Jennings plot. X-axis plots the time of measurement (e.g. day) and the Y-axis plot the measurement value for that unit of time. The lines denoting the mean value and 1, 2 and 3 standard deviations from the mean are explained in the figure.

concerned with identification of random error and within runs error detection [2, 7]. Other Westgard rules are focused on identification of systematic error and between runs error detection. In this chapter we will focus on the latter rules.

- 2_{2S} rule: The QC results are considered to have failed and a bias is present if two consecutive control values fall between the 3 standard deviations and 2 standard deviation limits on the same side of the means reference line.
- 4_{1S} rule: The QC results is considered to have failed and a bias is present if four consecutive control values fall on the same side of the mean reference line and are at least one standard deviation away from the mean.
- 10_x rule: The QC results are considered to have failed and a bias is present if 10 consecutive control values fall on the same side of the mean reference line.

These rules are shown in **Figure 3**.

2.3. Method comparison

Method comparison is used for initial assay validation as well as for studying accuracy of a test. The aim of method comparison is to establish whether the assay measures what it is supposed to measure and how accurately it measures it. The findings of method comparison also allow for correction of the results if a bias is found (i.e. calibration). The principal for method comparison is that a gold standard or a standard reference material exists where in the amount of analyte in the sample is exactly known (or known with a high degree of accuracy). We can use this reference standard as a comparator against the performance of our assay and determine the degree of bias that exists in our measurements. This essentially means that we are measuring the relative performance of our assay against the reference standard.

Ideally, identification of a bias should lead to a search for the source of the bias and systematic error, and attempts should be made to rectify the cause of the observed bias. However, there are instances in which no fault or solvable problem is identified; in these instances, if the assay has enough precision and stability as well as clinical merit then we can use the findings of method comparison to adjust for the observed bias.

Bias can take two general forms: constant bias and proportional bias. The constant bias is a difference between the observed measurement and the expected measurement that is constant throughout the range of the observations. Constant bias ($\beta 0$) is represented in regression statistics

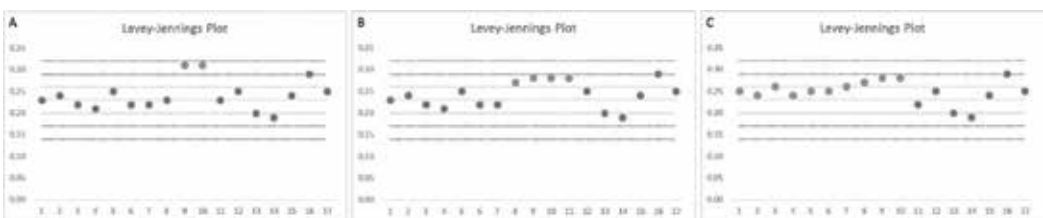

Figure 3. Examples of systematic error in Levey-Jennings plot: A. An example of 2-2S rule, B. An example of 4-1S rule, C. An example of 10x rule.

as intercept. Proportional bias ($\beta 1$), on the other hand, is proportional to the observed value of the measurement and varies across the range of measurements. Proportional bias is represented in regression statistics as the slope of the regression line. If the expected value of measurement is Y_i for each sample *i*, and the observed value of measurement for sample *i* is X_i, then we can form a linear regression between the expected values and observed values:

$$Y_i = \beta 0 + \beta 1\ X_i + \varepsilon_i \tag{3}$$

where ε_i is the random error of the expected observations under the Youden assumption which states that the random error of observed values is smaller than the random error for expected values.

The regression formula is the representation of the best regression line that shows the relationship of the observed value to the expected value. **Figure 4** shows the regression lines for different constant and proportional bias levels.

If no bias exists then $Y_i = X_i$.

The simple linear regression formula allows us to calculate the constant and proportional bias using a simple unweighted ordinary least squares estimator. In ordinary least squares (OLS) models, different candidate values for the parameter vector $\beta 1$ are tested to create regression lines. Then for each i-th observation the residual for that observation is calculated by measuring the vertical distance between the data point (Y_i, X_i) and the regression line formed using the candidate value. The sum of squared residuals (SSR) is determined as a measure of the overall model fit. The candidate value that minimizes the sum of squared residuals is considered as the OLS estimator for the slope. For simple method comparison studies where only two comparators are present the model can be simplified as:

$$\beta 1 = \frac{\sum X_i Y_i - \frac{1}{n}\sum X_i \sum Y_i}{\sum {X_i}^2 - \frac{1}{n}\left(\sum X_i\right)^2} = \frac{Covariance(X, Y)}{Variance(X)} \tag{4}$$

The constant bias can be calculated by subtracting the mean expected value from mean observed value weighted by proportional bias:

$$\beta 0 = \overline{Y} - \beta 1\ \overline{X} \tag{5}$$

Constant and proportional bias usually has different root causes. Constant bias often stems from insufficient blank sample correction and is fairly easy to address and rectify. Proportional

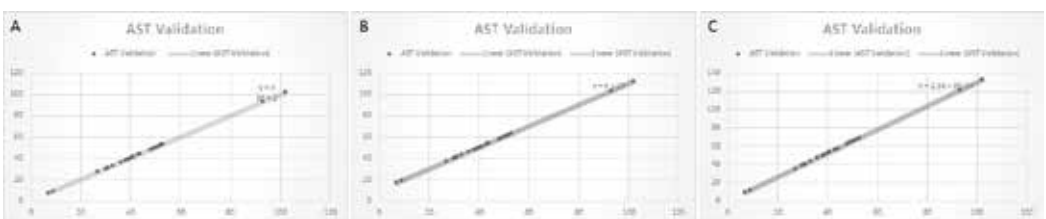

Figure 4. A. When no systematic error exists. B. Shows constant bias. C. Shows a proportional bias.

problems can sometimes be caused by the difference in the composition of calibrator samples and the standard samples or biologic test matrices. The matrix of the reference standard is usually near the actual matrix of the patient samples and thus may contain confounders which may adversely affect the measurement. Yet calibrators often do not have a biologic matrix. If the source of the proportional bias is due to calibration problems, then a recalibration can rectify the problem.

The problem with the Youden assumption is that it considers our observations to have no random disruptions, an assumption which is false as we know every measurement is associated with a degree of uncertainty and imprecision. Alternatively, we can use Deming's regression where the random error for both expected and observed values is factored into the calculation of the proportional and constant bias. In Deming's regression a ratio of the variances of the random error of observed and expected values is calculated:

$$\delta = \frac{{\sigma_\varepsilon}^2}{{\sigma_\eta}^2} \tag{6}$$

where ${\sigma_\varepsilon}^2$ is the variance of the expected values random error and ${\sigma_\eta}^2$ is the variance of the observed values random error. Using this ratio, the OLS estimator for the proportional bias can be given by:

$$\beta 1 = \frac{(Var(Y) - \delta Var(X)) + \sqrt{(Var(Y) - \delta Var(X))^2 + 4\delta Covar(X,Y)^2}}{2Covar(X,Y)} \tag{7}$$

This regression formula is also known as the maximum likelihood estimator [9].

If a linear relation between errors and measurements exists (or is assumed) then an alternative method for error detection is to create Bland-Altman plots. In these plots, the average of the paired values for expected and observed values is plotted on the x-axis and the difference of each pair is plotted on the y-axis. In this method the average difference of the values is called bias and the standard deviation of the differences is also calculated to determine the limits of agreement which constitutes Mean difference ±1.96SD.

The Bland-Altman approach allows for a visual inspection of the proportional bias. However, by dividing the limits of agreement by the mean value of the expected values we can obtain a metric called percentage error. The acceptable percentage error levels for different analytes have been determined and are standardized. In cases where the percentage error exceeds the acceptable levels, corrective action is needed for the detected bias [10].

2.4. R statistics

One of the important statistics for simple linear regression is calculation of the Pearson's r coefficient. This coefficient shows how well the compared results change together and can have values of between minus 1 and 1. This coefficient can be calculated by dividing the covariance of the two variables to the product of their standard deviations:

$$r = \frac{Covar(X, Y)}{\sigma_X \sigma_y} \tag{8}$$

The closer the r coefficient gets to 1, the greater the linear relationship is between the two variables. Some interpret the r coefficient as a measure of correlation with r coefficients more than 0.8 showing correlation. However, in laboratory medicine a correlation of 0.8 actually signifies a great degree of bias. In fact, laboratories should aim for a perfect degree of linearity (r > 0.99) to ensure that systematic error is minimized. Attaining a Pearson's r coefficient of <0.975 signals the presence of systematic error and should prompt the lab to conduct further investigation (using t-test and f-test) to determine the source of this error.

The degree of agreement or the coefficient of determination (R^2). This coefficient is calculated from the ratio of explained variance to the total variance of Y:

$$R^2 = \frac{\sum \left(\widehat{Y}_i - \overline{Y}\right)^2}{\sum \left(Y_i - \overline{Y}\right)^2} \tag{9}$$

where $\widehat{Y}_i$ is the calculated value of Y based on the regression for the i-th observation and Y_i is the actual value of Y for i-th observation.

Alternatively, the coefficient of determination can be simply calculated by squaring the Pearson's r coefficient. While the Pearson's r coefficient shows the presence of linearity, the coefficient of determination helps us to determine how well the regression line fits the actual data points. In assessment of a method comparison evaluating this coefficient is necessary as it shows fit of the model: The closer the coefficient gets to 1, the better the regression line fits actual data points. However, it must be noted that even at numbers very close to 1 significant bias may exist. For example, a 5% bias will only result in a R squared score of 0.99 and a 10% bias will result in a R squared score of 0.96. For laboratory medicine purposes we should aim for a R squared score of more than 0.99.

2.5. T-test and F-test

In cases where there is a suspicion of significant bias (as determined by Pearson's r or R squared statistics), then we should determine whether the bias stems from difference in the mean assay concentration or in the variance of the assay. To check for mean we run a paired t-test, and, to check for variance, we run an f-test.

The paired t-test is performed by comparing the means of the observed and expected values; more specifically the mean difference of the values (μ_D) is used for the comparison. The t-statistics can be calculated by:

$$t = \frac{\mu_D}{\sigma_D / \sqrt{n}} \tag{10}$$

where n is the number of data points and σ_D is the standard deviation of the mean difference. To determine the significance of the results (the p-value), the t-statistics should be looked up on a t table corresponding the degree of freedom; the degree of freedom in paired t-tests equals n–1.

A t-test with a significant p-value signifies the presence of a significant bias in the mean of the methods. The next step then would be to determine whether the systematic error represents a constant bias or a proportional bias. This can be done by examining the regression curve or equation. The presence of an intercept signifies a constant bias while presence of a slope other than 1 signifies proportional error. The correction for a constant bias is simple and would require adding the constant to the measurement results. Correction of the proportional bias, however, requires a recovery experiment as described in Section 3.8 below.

The f-test compares the expected variance of the values to the observed variance; while the t-test compares the centroid of the data points (the mean), the f-test deals with the distribution and variance of the data points (the variance). The t-test is more sensitive to differences in the values in the middle of the data range while f-test is more sensitive to differences in the extremes of the data range. A significant f-test would signify random error in the measurement or in other words imprecision. To calculate the f-test the following equation is used (the larger of the two variances will always be the numerator and the smaller one the denominator in this fraction):

$$f = \frac{Var_1}{Var_2} \tag{11}$$

The degree of freedom of the f-test is (n-1, n-1) and the significance threshold can be looked in a f-table corresponding the degree of freedom.

It is important to perform the f-test prior to the t-test; one of the basic assumptions of the t-test is that the standard deviations of the data points are similar between the two groups, i.e. no significant imprecision should exist for t-test results to be valid. In presence of a significant imprecision, the determination of presence of a significant bias should be done using a Cochran variant of the t-test.

In Cochran variant of t-test, standard deviation cannot be pooled between the two groups:

$$t = \frac{\mu_D}{\sqrt{\frac{Var_1 + Var_2}{n}}} \tag{12}$$

The critical value for the t-statistics should also be calculated:

$$Critical\ t = \frac{\frac{t}{n}(Var_1 + Var_2)}{\frac{Var_1 + Var_2}{n}} \tag{13}$$

where t is the t-score corresponding to n-1 degrees of freedom [11].

2.6. Accuracy profile

Accuracy profiling has moved away from treating bias and imprecision as separate entities. In fact, most guidelines (whether based on the total error principles or measurement uncertainty principles) combine bias and imprecision for acceptability criteria. To calculate bias and

imprecision, we need to run a reproducibility study. Reproducibility of quantitative studies is obtained by repeated measurements of a sample in a series and then conducting multiple series of reproducibility studies.

The overall measurement of bias will be the difference between the mean value of the analyte obtained from the repeated measurement and the reference value:

$$Bias = Overall\ mean - Reference\ value \tag{14}$$

Bias and imprecision are used to form the tolerance interval; it is the interval which, with a determined degree of confidence, a specified proportion of results for a sample fall. Tolerance interval can be expressed as:

$$Tolerance\ Interval = reference\ value + bias \pm intermediate\ precision \tag{15}$$

For laboratory medicine, the tolerance interval of analytes needs to be smaller than the acceptability limits. In united states, the acceptability limits are set and governed by the Clinical Laboratory Improvement Amendments of 1988 (CLIA88). These acceptability limits are provided under the following heading: 42 CFR Part 493, Subpart I - Proficiency Testing Programs for Nonwaived Testing (https://www.gpo.gov/fdsys/pkg/CFR-2011-title42-vol5/pdf/CFR-2011-title42-vol5-part493.pdf).

The important factor from intermediate precision that is needed in calculation of tolerance interval is the standard deviation of reproducibility (S_R). The standard deviation of reproducibility can be calculated by the following equation:

$$S_R{}^2 = \frac{1}{n}\left(\frac{Var_{betweenseries}}{p-1} + (n-1)\frac{Var_{withinseries}}{n-p}\right) \tag{16}$$

where n is the number of within-series measurement repeats and p is the number of series of reproducibility measurements.

An advantage of calculating the intermediate precision is that we can use it in combination with within- series repeatability to determine the uncertainty of bias:

$$Uncertainty\ of\ Bias = 1.96\left[\frac{n\left(S_R{}^2 - S_r{}^2\right) + S_r{}^2}{np}\right]^{1/2} \tag{17}$$

$S_r{}^2$ is the within-series repeatability and can be calculated using the following equation:

$$S_r{}^2 = \frac{Var_{withinseries}}{p(n-1)} \tag{18}$$

Uncertainty of bias is essentially 1.96 times the standard deviation of bias which corresponds to a 95% confidence interval for bias determination.

The between-series reproducibility is calculated using the following equation:

$$S_L{}^2 = \frac{1}{n}\left(\frac{Var_{betweenseries}}{p-1} - S_r{}^2\right) \tag{19}$$

The between-series reproducibility is used in calculation of the Mee factor (K_s). Mee factor is the other component of intermediate precision. Since the calculation of the Mee factor is complicated we have broken it down into a series of equations. The first step is to calculate the H ratio:

$$H = \frac{S_L{}^2}{S_r{}^2} \tag{20}$$

The next step is to calculate the G^2:

$$G^2 = \frac{H+1}{nH+1} \tag{21}$$

Which in turn is used to calculate C:

$$C = \left(1 + \frac{1}{npG^2}\right)^{1/2} \tag{22}$$

The final step is to multiply C by the t-score associated with the degree of freedom (dof):

$$Degree\ of\ Freedom = \frac{(H+1)^2}{\frac{\left(H+\frac{1}{n}\right)^2}{p-1} + \frac{1-\frac{1}{n}}{np}} \tag{23}$$

And:

$$K_s = C \times t_{dof} \tag{24}$$

By calculating the Mee factor and the standard deviation of reproducibility we can now obtain the intermediate precision:

$$Intermediate\ precision = K_s \times S_R \tag{25}$$

Thus, we can rewrite the tolerance interval as [12]:

$$Tolerance\ Interval = reference\ value + bias \pm (K_s \times S_R) \tag{26}$$

2.7. Weighting procedures

The problem with simple linear regression is that is based on a set of assumptions; one of the problematic assumptions is that the standard deviation of the random error is constant throughout the range of measurement. This assumption, however, is often wrong as the standard error of measurement is often much larger near the extremes of measurement range (near the limit of detection and the highest range of linearity). The solution in laboratory

medicine can be to run linearity experiments and limit the measurement range based on the linearity results. Despite this the effect of random variation on the regression line remains. To rectify this, a solution is to employ a weighting procedure.

The simplest weighting procedure is to use the standard deviation of variation for each data point of the method comparison study. This requires that the method comparison study is repeated multiple times (20-30 times). This allows us to calculate the standard deviation of measurement for each point (S_i). The weighting coefficient will then be the inverse of this standard deviation:

$$w_i = \frac{1}{S_i} \tag{27}$$

This weight can then be incorporated into the equations of the method comparison. For example, the r coefficient can be recalculated as:

$$r = \frac{\sum w_i\left(X_i - \overline{X}\right)\left(Y_i - \overline{Y}\right)}{\left(\sum w_i\left(X_i - \overline{X}\right)^2 \sum w_i\left(Y_i - \overline{Y}\right)^2\right)^{\frac{1}{2}}} \tag{28}$$

Weighting can often considerably decrease the bias percentage especially at the extremes of measurement compared to non-weighted regression. Weighting by inverse of standard deviation tends to normalize the relative bias at the extremes of measurement while weighting by inverse of variance tends to favor the bias correction for lower ends of measurement (less bias at lower concentrations). The decision for weighting and/or choice of weighting procedure should be based on the assay characteristics and performance requirements [13].

2.8. Recovery percentage

To estimate the proportional bias, a recovery experiment is needed. The recovery experiments are performed by calculating the amount of recovery when adding a known amount of the analyte to the sample: this is done by dividing the measurement sample into two equal aliquots and performing the measurement for both aliquots. To one of the aliquots, a known amount of target analyte is added (aliquot 1). For the other aliquot (aliquot 2) an equal amount of diluent is added and the measurement is repeated. The recovery percentage can then be calculated:

$$Recovery\% = \frac{(Analyte\ amount\ in\ aliquot\ 1) - (Analyte\ amount\ in\ aliquout\ 2)}{Amount\ of\ analyte\ added\ to\ aliquote\ 1} \times 100 \tag{29}$$

The recovery or bias percentage is often used in laboratory medicine to state the proportional bias. Most of the regulatory agencies have set critical values for the recovery percentage for different analytes. The advantage of using recovery percentage is that it normalizes to 100 allowing for easier understanding of the scale of bias present [2].

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3. Bias detection without comparators

Up to this point we have discussed bias detection methods that use a reference material or comparator to assess the presence of bias. While this has been the accepted standard for many laboratory regulatory agencies, there are arguments against this approach to bias detection: first of all, the assumption of method comparison studies is that the reference material (control samples) values are true and do not suffer from imprecisions. The measurement uncertainty is considered to be minimal in these samples. Yet, unless these samples vary considerably from the biologic sample matrix, a degree of measurement uncertainty would exist in these samples which lead to inaccurate estimates of bias and imprecision of laboratory instruments and techniques. On the other hand, running repeated control samples with each run and the need for revalidation of the instrument and techniques after each change in the parameters, requires a considerable investment in terms of time, labor and cost.

Alternatively, the systematic error can be determined by using the patient samples. This can be done by either tracking the results of known normal patients (i.e. those expected to have a result within the reference range based on their clinical and physiologic state) or by following the trend of all the results of an analyte over time. Using patient samples has the advantage of including the inherent biologic uncertainty into the calculation of bias.

3.1. Average of normal (AON)

In this approach the comparator for quality control would the average values of the analyte in normal individuals. This requires us to know the population average and standard deviation for that analyte. If we measure the analyte in a normal individual, we would expect the results to approximate the population average. Deviations of the normal results from the expected reference normal can signal the presence of a systematic error.

In AON, the mean value of normal samples is compared to a mean reference value. The mean reference value should be established by the laboratory based on the population it serves; this is best done as part of the initial validation of an assay when a large size sample of normal individuals is tested to establish the reference ranges. This experiment allows us to calculate the population mean, standard deviation and standard error (SD/√N). We expect the Average of Normals from our analytical run to fall within the 95% confidence interval of the population mean.

$$95\%CI = \textit{Population Mean} \pm 1.96\ \textit{Standard Error} \tag{30}$$

With each analytical run, a sample of normal results should be used to calculate the Average of Normals for that analytical run. If the calculate average is beyond the 95% CI of the population then we have detected a systematic error in the analytical run.

In AON method, as the size of the normal sample increases the probability of detecting bias also increases. The size calculations for the AON method are determined by the ratio of the biological variance of the target analyte (CVb) to the variance of the method (CVa) (CVb/CVa)

as well the expected probability of detecting the bias. To help with these calculations, one can utilize the Cembrowski nomogram [14] or, alternatively, the methods used in [15]. It is also possible to perform the AON by performing a two-sample independent t-test.

3.2. Moving patient averages

Unlike the AON method, in moving patient averages, all the results of an assay are included in evaluation of bias. The principle for moving patient averages is that the samples tested in a laboratory follow a repeating pattern. This assumption means that the overall biologic and clinical spectrum of patients and individuals tested in the laboratory is constant throughout the analytical runs. In moving patient averages, we expect the average results of an assay for two overlapping subsets of patient to be constant. In this method, for example, an average is calculated on the first 100 patients, should be similar to the average calculated based on the results of patients number 2 to 101, etc.

The moving average can be calculated using exponentially weighted moving average ($\overline{X}_{M,i}$). It is important to consider that, in moving patient averages the weight $(1-r)$ assigned to previous results average ($\overline{X}_{M,i-1}$) should be greater than the weight (r) assigned to the most recent results ($\overline{X}_i$) (in other words the average of each batch is weighted down by previous averages). This can be stated as:

$$\overline{X}_{M,i} = r\overline{X}_i + (1-r)\overline{X}_{M,i-1} \tag{31}$$

The weight assigned to current values is usually set between 0.05 and 0.25 with recommended value of 0.1.

The comparator in moving patient averages are the control limits. We expect the weighted patient average to fall within the control limits for that test. Any moving patient average outside of this control limit signifies the presence of a bias. The control limit equation is provided below.

$$\textit{Control limits of exponential moving average} = \overline{X}_{M,0} \pm L\sigma\sqrt{\left|\frac{r}{2-r}\left[1-(1-r)^{2i}\right]\right|} \tag{32}$$

where L is a constant set based on the confidence level (for 95% CI, L equals 2), and σ is the standard deviation of the current batch.

The moving patient averages can also be evaluated using the Bull's algorithm. In this approach, the moving average ($\overline{X}_b$) is calculated for subsets of 20 samples with 19 patient values and one value representing the previous moving average. These values are weighted differently (i.e. more weight is assigned to the previous moving average than the 19 new samples).

The general formula for Bull's moving average can be written as:

$$\overline{X}_{b,i} = (2-r)\overline{X}_{b,i-1} + rD \tag{33}$$

where $\overline{X}_{b,i}$ is the current moving average, r is the weight for current values (with possible values of $0 < r \leq 1$, usually set to 1), $\overline{X}_{b,i-1}$ is the previous moving average and D is calculated

from the value of current measurements in the batch. If we assume a value of 1 for r then we can write the bull's algorithm as:

$$\overline{X}_{b,i} = \overline{X}_{b,i-1} + \left(\frac{\sum_{j=1}^{N} \sqrt{X_j - \overline{X}_{b,i-1}}}{N} \right)^2 \tag{34}$$

where N is the number of results in the batch.

The control limits of Bull's moving average are set as $\overline{X}_{b,0} \pm 3\%\overline{X}_{b,0}$, with $\overline{X}_{b,0}$ being the target value for that analyte.

The advantage of moving averages is that they can filter out outliers' effect thus removing confounding by imprecision.

The moving patient averages algorithms are very powerful for detection of bias: they can routinely identify bias percentages of 1% and more. Most automated hematology analyzers use moving patient averages to check for presence of bias in their assays. However, the patient moving averages algorithms have suffered from implementation problems and are not widely used beyond hematology analyzers [2].

3.3. Time series analysis and forecasting for bias identification

An extension of the moving patient averages is the application of time series analysis and forecasting for bias detection. In time series analysis the previous trends of the analyte results are used to predict (forecast) the trend in future. If the observed analyte results deviate from the forecasted trend, then a measurement error may exist. In the setting of laboratory medicine, we need to be able to detect bias in short time series and distinguish the measurement error from the noise and chaos stemming from biologic variation. Here, we will introduce the concept of using time series analysis for bias detection but we will not explain the methodology in depth as it goes beyond the scope of this chapter.

In forecast models, a series of data points are used to create one or more projection patterns for future trends. This is done using forecasting models such as ARIMA (Autoregressive integrated moving average). These projections are often correct for very short-term predictions (next 1 or 2 data points), but for forecasting further, the noise and chaos cause the prediction accuracy to fall. However, by examining the correlation of predicted and observed values and documenting its changes as we forecast further into the future, we can determine if the observed pattern represents the deterministic chaotic nature of biologic measurement or if it represents a measurement error; for measurement error we expect the correlation coefficient to remain constant with time; however, with chaos, we expect the correlation coefficient to deteriorate over time [16].

There are other approaches using times series analysis that can be helpful in systematic error identification. One of these approaches uses unit root tests such as the Dickey-Fuller test [17]. These tests examine whether a time series is stationary over time, i.e., whether the mean and

variance are constant over time. In contrast, nonstationary time series will have either a varying mean and/or varying variance over time. Using this approach any departure from stationarity can signal either a drift (proportional bias) and/or a shift (constant bias) or even increase in imprecision over time (difference-stationary nonstationarity) [17]. If the Dickey-Fuller test returns a significant p-value then we can say that the series is stationary, and no significant measurement error is present.

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Chapter 6

Understanding Quality Control with Urinary Iodine Estimation

Husniza Hussain and Rusidah Selamat

Additional information is available at the end of the chapter

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Abstract

Urinary iodine is a tedious assay since it is easily evaporated. A quality system is needed to maintain quality control in a urinary iodine laboratory. In this chapter, a quality system for the urinary iodine micromethod (UIMM) had been discussed briefly. The system covers the pre-analytical, analytical and post-analytical stages of the assay. Each stage depends on each other to complete the whole quality system which ensures the validity of the laboratory results. The laboratory procedures, started with method validation, are very important to be adhered strictly. The internal quality control (IQC) in every analysis and participation in External Quality Assurance (EQA) program will ensure validity of assay and will compare laboratory performance to the others. Evaluation from time to time using Sigma metrics is also vital to complete the quality system as troubleshooting and corrective actions taken will improve the UIMM from time to time. These are supported by the documents and records. A good quality system will guide the urinary iodine analysis operators to gain confidence in their work and the results they obtain for the respondents in monitoring elimination program of iodine deficiency disorders (IDD).

Keywords: urinary iodine, quality control, urinary iodine micromethod, sigma metrics, iodine deficiency disorders

1. Introduction

Iodine deficiency disorders has been one of the targets for elimination by the World Health Organization (WHO) throughout the world. It is a nutritional related disease which is preventable through adequate iodine supplementation. Iodine facilitates optimal brain development

in fetuses and it is involved in the synthesis of thyroid hormones, one of the vital hormones in human body. Hence, with optimum iodine supplementation, it could lead towards more intelligent population.

Urinary iodine is the test in determining the baseline of a population's iodine nutrition before decision of implementing Universal Salt Iodization (USI) is made. It is also important to monitor the iodine nutrition of the population after USI has been implemented. Sampling for urinary iodine testing among school children is non-invasive and urinary iodine is a reliable biomarker for immediate iodine level in one's body. Although thyroglobulin is the biomarker for long-term iodine nutrition in a human, urinary iodine remains the chosen biomarker for the purpose of easier and cheaper way of estimation of iodine nutrition status worldwide. In ensuring the validity of urinary iodine tests results, quality control has to be implemented in the laboratory. In the subsequent sections of this chapter, the quality control plans and implementation are discussed for the benefit of urinary iodine laboratory managers and operators.

1.1. Iodine deficiency disorders (IDD)

Iodine deficiency disorders (IDD) can cause delayed brain development, stunting and stillbirth, and affects humans throughout their life. IDD has been a focus for elimination by the World Health Organization (WHO) since it is a preventable disease through intervention of adequate iodine nutrition. Human residing in the mountain areas are prone to be iodine deficient since iodine is swiped down by rainfall towards the sea [1]. IDD is segregated into severe, moderate, mild IDD. Examples of symptom are goiter, retardation & cretinism (**Figure 1**). The iodine nutritional status of a population is usually determined from median urinary iodine of schoolchildren aged 8–10 years old [2]. Elimination of IDD may reflect the growth of more

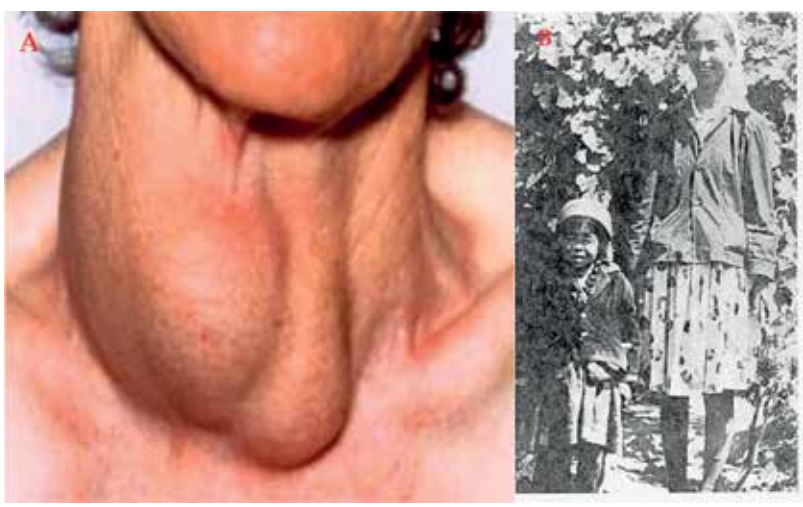

Figure 1. Iodine deficiency disorders (IDD) symptoms, e.g. (A) goitre & (B) cretinism [image reproduced with permission of the rights holders, www.drsosha.com & Human Info NGO; credit is given to United Nations Administrative Committee On Coordination (Sub-Committee On Nutrition)].

intelligent generation to come since it was reported that babies with higher intelligent quotient (IQ) were born from mothers with adequate iodine nutrition during pregnancy [3]. Iodine deficiency may also affect the production of thyroid hormones since each of them need iodine to be covalently bound to the tyrosine backbone [4]. Lack of thyroid hormone production may lead to hypothyroidism and may affect many metabolisms in human body [5]. Thus, iodine is very vital to human growth and development.

1.2. IDD elimination program

IDD elimination program is carried out worldwide. Various interventions of iodine have been implemented including through iodized water and iodized salt. Intervention of iodized salt is the most cost-effective strategy in the elimination program. Iodized salt interventions require only investment of 5–10 cents/year per person [6]. In 20 years, iodization of salt had reduced the prevalence of IDD, whereby, in the year 1993, the number of 131 iodine-deficient had been reduced to only 31 countries in the year 2014. In 2014 also, 70% households had access to adequately iodized salt. Within the years 2009–2013, it was estimated that 50–86% of households are consuming adequately iodized salt, ranging from the least developed countries to East Asia and Pacific countries [7].

1.3. IDD monitoring and determination of median urinary iodine of population

Urinary iodine testing is mandatory before any intervention implementation and in monitoring the universal salt iodization (USI) programs. Urinary iodine laboratories are responsible to perform the urinary iodine testing on respondent samples in determining the population median values. Even though the target group to determine median urinary iodine is schoolchildren aged 8–10 years old, various researches were also done on pregnant women and followed up with their babies to investigate the relationship between iodine status during pregnancy and the babies' IQ [3]. Thus, quality control throughout the laboratory performance is of utmost importance to ensure that the results obtained are valid and reliable to generate accurate reports.

2. Quality in urinary iodine laboratories

2.1. Quality management system

The urinary iodine laboratories (there are five laboratories in the country) were formed under the National IDD Eradication Program parallel with the initiative by the World Health Organization (WHO) for eliminating IDD worldwide. Quality management has been practiced throughout the Ministry of Health following the twelve elements outlined by WHO [8] (**Figure 2**). However, in this chapter, only the quality control aspects related to the urinary iodine laboratory quality management will be discussed, mainly on the processes related to the analysis.

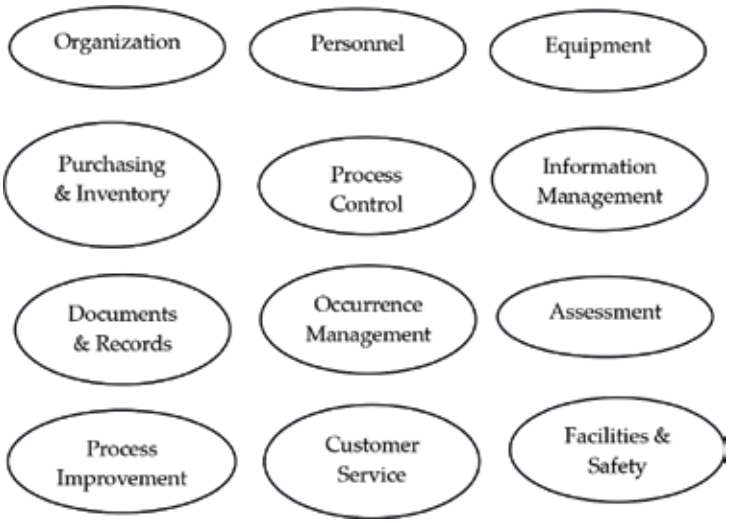

Figure 2. The 12 elements of quality management outlined by WHO [8].

2.2. Quality system in a urinary iodine laboratory

2.2.1. Method

Following the Quality System (**Figure 3**), it started with method modification done in 2004 with migration from performing test wholly in test tubes to performing test half-way in test tubes (during sample digestion) and half-way in 96-well microtiter plates (during reagent mixing and absorbance reading). Method validation was done by the Institute for Medical

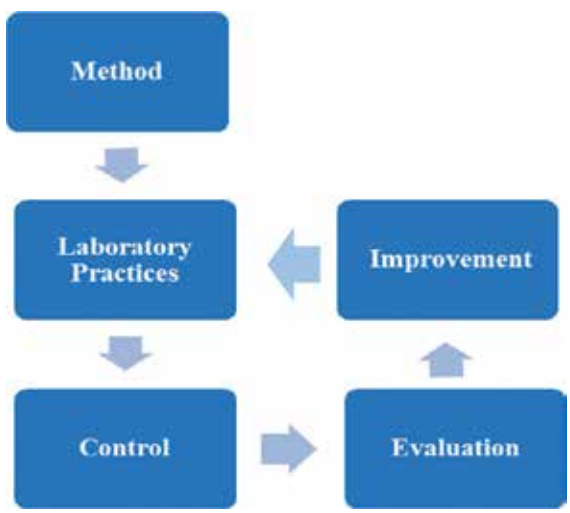

Figure 3. Flowchart of Quality System in the urinary iodine laboratory at the Institute for Medical Research (IMR), Malaysia.

Research from the year 2005 until 2006, comprised of sensitivity, precision, linearity, recovery and method comparison [9].

2.2.2. Laboratory practices

Training is important to enhance skills and competency in performing laboratory work. Maintenance of instruments is vital to ensure adequate heating during sample digestion, correct pipetting of samples and reagents, and accurate absorbance reading. Another important precaution to be made is to avoid contamination from salt iodine laboratory and unclean glassware. Since iodine is easily evaporated, iodine in salt which are usually present in parts per million (ppm) can be dispersed in the air in the same room environment and interfere with urinary iodine measurement which is in parts per billion (ppb). Inadequate cleaning of glassware may cause false detection of high concentration of iodine in urine. Reagents shelf-life should also be abided strictly as aged reagents may cause internal quality control (IQC) values to be out of limits.

2.2.3. Control

IQC sample preparation follows the order of %CV ≤ 20% for Low control, ≤15% for Medium control and ≤10% for High control, in obtaining allowable ranges (mean ± 2SD) from replicates of samples of n ≥ 20. The order of %CV set for the laboratory superseded the % CV set by TUIQP, previous EQA program for urinary iodine which set %CV of 20% for all Low, Medium and High control ranges. Each control level (Low, Medium or High) should be included in the assay with minimum replicate of n = 2. External Quality Assurance (EQA) was done once or twice/year (2006–2009) and is currently being done for three cycles/year (2010-present). Current EQA program provides four concentration levels of samples and requires to be assayed in duplicates in three independent assays (n = 6).

2.2.4. Evaluation

Evaluation is performed from IQC and EQA results. IQC results are obtained from every assay while EQA results are obtained from every cycle of the program. Laboratory performance was also determined by conducting evaluation using the Six Sigma quality metrics which focused on the laboratory achievement as compared to the world-class level of Sigma-6 [10]. Evaluation should be done periodically.

2.2.5. Improvement

Corrective actions are made upon every occurrence of non-conformance. Corrective actions are meant to troubleshoot problems and prevent them from being repeated. Relevant IQC rules [11] are to be obeyed and corrective actions are done accordingly to improve quality of test. Improvement may lead to better laboratory practices and the cycle of the Quality System (**Figure 3**) continues as it gets better throughout time.

A well-managed laboratory quality system will enable good laboratory practice, assessment of method, instruments and laboratory performance, and will help the interpretation of respondent results by knowing the accuracy of the method used for Urinary Iodine measurement.

In defining the scope of method, all method validation data should be noted [9] including the expected precision and accuracy and method robustness. The type of equipment to be used as listed in the instrument maintenance section should be noted. The method is applicable to all laboratories possessing the three main instruments, i.e. the heating block, microplate reader, single channel and multichannel micropipette. It is also important that the heating blocks are placed in a fume hood during sample digestion at 100°C for 1 hour so that any fume accumulated can be channeled out from the laboratory for safety purposes. Another vital issue is the skills and competency of the operators which determine the high precision and accuracy of results especially on the pipetting which ensures excellent replicates.

2.3. Urinary iodine analysis

2.3.1. Urinary iodine micromethod

Urinary iodine is a biochemical indicator in monitoring iodine deficiency disorders (IDD) [12]. Urinary iodine is measured using the urinary iodine micromethod (UIMM) which was modified to improve method used in the urinary iodine laboratories in the country [9]. The modified method offers minimal expenditure for new devices, usage of less hazardous chemicals and lesser amount of chemical waste produced. Through method validation, comparison plot and difference plot [11] had been prepared for UIMM against the urinary iodine measurement method proposed by the World Health Organization (WHO) [12]. From the comparison plot (**Figure 4**), we are ensured that the UIMM works well and it is comparable to the WHO method with excellent correlation coefficient (r) of 0.9428. From the difference plot (**Figure 5**), the performance of UIMM is shown with not much difference from the WHO method with

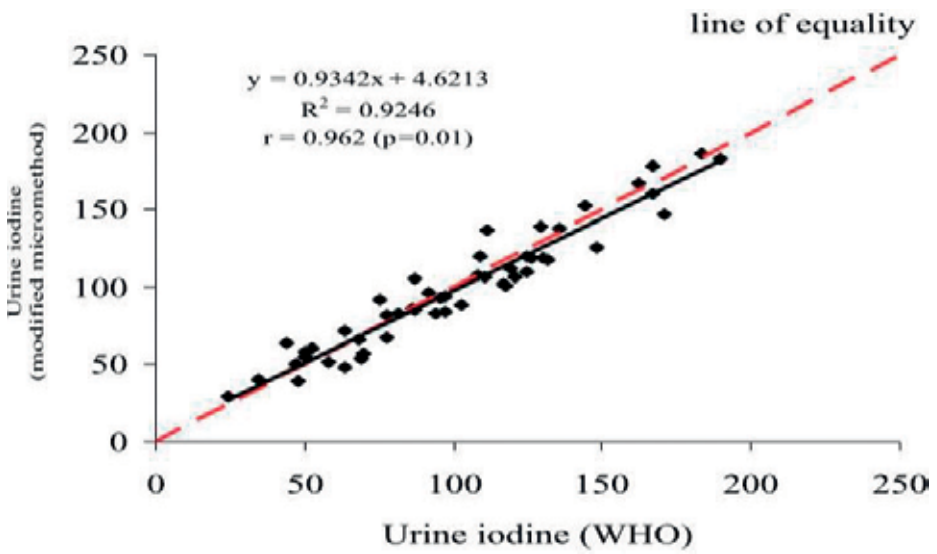

Figure 4. Comparison plot of the modified method versus WHO method (image reproduced with permission of the rights holder, Tropical Biomedicine).

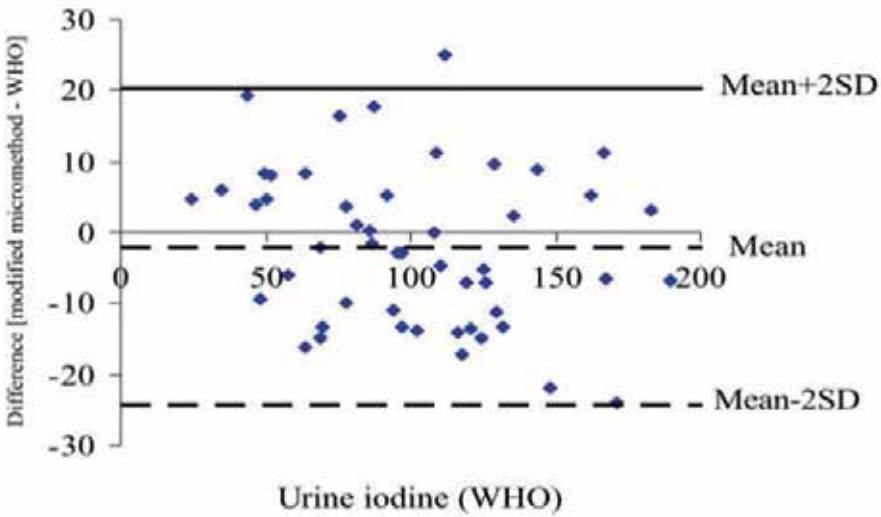

Figure 5. Difference plot between the modified method and the WHO method (image reproduced with permission of the rights holder, Tropical Biomedicine).

only two out of 50 readings with biases of more than ±22 μg/l. Other method validation includes (i) sensitivity: 13.809 μg/l, (ii) intra-assay precision: 5–13%, (iii) inter-assay precision: 7–15%, (iv) linearity: correlation coefficient (r) = 0.993, and (v) recovery: 106–114% [9].

Mainly, there are three main solutions used in the UIMM, namely ammonium persulfate, arsenious acid and ceric ammonium sulfate solutions (**Table 1**). The former oxidizes the urine samples and the two latter solutions contribute to the execution of the Sandell-Kolthoff reaction.

The main steps in the UIMM are sample digestion and Sandell-Kolthoff reaction [reaction formulas (1) and (2)]. Urine digestion eliminates the interferences which may cause false positive in the analysis [13]. Arsenite in the presence of iodine reduces yellow-colored ceric ions to colorless cerous ions. Thus, by spectrophotometrical measurement, the absorbance is inversely correlated with the concentration of urinary iodine.

Urinary iodine determination incorporation of two steps of action, i.e. urine digestion at high temperature and iodine measurement in Sandell-Kolthoff reaction of:

$$As^{3+} + I_2 \rightarrow As^{5+} + 2I^- \quad (1)$$

$$\underset{(yellow)}{2Ce^{4+}} + 2I^- \rightarrow \underset{(colorless)}{2Ce^{3+}} + I_2 \quad (2)$$

The procedural steps for UIMM comprised of four steps as depicted in **Figure 6** [9].

Successful analytical procedures are supported by good pre-analytical (involves documents, chemicals, consumables and glassware) and post-analytical processes (involves records and reports). These processes are discussed further in the subsequent subsections.

Chemicals	Purpose
Ammonium persulfate	Digestion
Arsenious acid solution (As_2O_3, NaCl, H_2SO_4)	Adding As^{3+} ions
Ceric ammonium sulfate, H_2SO_4	Adding Ce^{4+} ions

Table 1. Purpose of each chemical addition in the urinary iodine micromethod (UIMM).

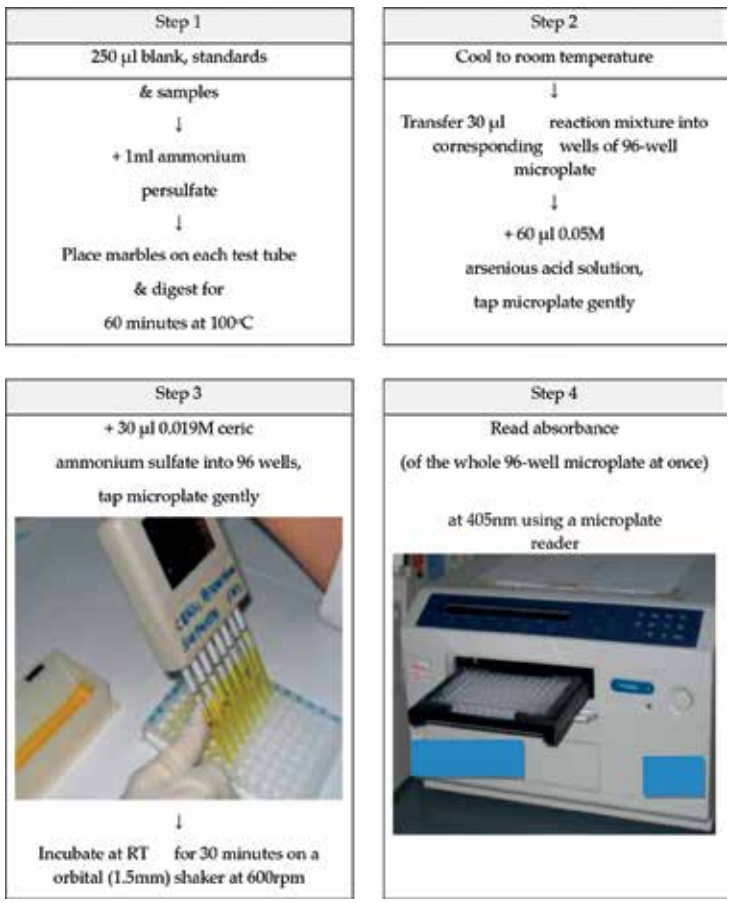

Figure 6. Diagram showing steps in UIMM assay.

2.3.2. Chemicals, consumables and glassware

Chemicals to be used for urinary iodine measurement should be more than or equivalent to analytical reagent-grade. Consumables to be used are non-sterile while the 96-well microtiter plates can be used either of flat- or round-bottom polystyrene ones. Microtiter plate lids should be covered with aluminum foil to prevent direct light onto reaction mixture. Sandell-Kolthoff reaction is sensitive to heat [14]. Thorough washing of glassware (test tubes, volumetric flasks,

beakers, glass marbles) is required to avoid carryover of the leftovers of iodine in the glassware into subsequent assay. Current practice of soaking glassware in distilled water overnight for two consecutive days after washing with detergent is adequate to remove iodine residues and to ensure cleanliness for usage in the next assay.

2.3.3. Maintenance of equipment

2.3.3.1. Heating block

During the digestion procedure of the urinary iodine assay, it is essential that heat be distributed evenly across the heating block on every test tube. Check for even heat distribution can be achieved by placing twenty test tubes filled with 2 ml oil or sand and inserted with a thermometer each. Heating block should achieve 100°C before placing the test tubes and heated for 20 minutes. Temperature of each thermometer should be recorded and mean ± SD is calculated. The temperatures recorded across the digestion unit should fall within the manufacturer's stated temperature distribution range. CV should be ±5%.

2.3.3.2. Microplate reader

The uniformity absorbance reading of the microplate reader maintenance checking is done by pipetting 200 μl of 1:1500 green food coloring in water in the first row (Row A) of a 96-well microtiter plate. Mean, SD, 1.5SD and CV of the readings are then determined. A scatter plot should be graphed for the individual readings with horizontal lines for mean and mean ± 1.5SD. The number of readings outside mean ± 1.5SD is determined and its percentage is calculated. Percentage error should be ≤20%. If it is not achieved, the maintenance check should be repeated and a request for calibration or repair should be lodged if problem persists. Maintenance check up every three-monthly has to be performed.

2.3.3.3. Micropipette

To check the micropipette performances, a maintenance-check-up every three-monthly is performed. Three points of volume should be tested, i.e. within the lowest, middle and highest ranges. For example, if the micropipette volume range is 100–1000 μl, then the pipette should be checked at 100 and next time around at 500 or at 1000 μl; it is up to the operator to decide. Water with the chosen volume should be pipetted into 10–20 clean disposable test tubes (LP3 or LP4 tubes). The weight of the tubes with and without water is recorded accordingly. Other information that should be recorded includes the brand and model of pipette, its code number, date of maintenance check-up and name of the person carrying it out. Mean, standard deviation (SD) and coefficient variation (CV) of the readings are determined. Inaccuracy is also determined as follows:

- % Coefficient of Variation (CV) = $\frac{\text{SD}}{\text{Mean}} \times 100\%$
- % Inaccuracy = $\frac{(\text{calculated mean} - \text{set volume})}{\text{Set Volume}} \times 100\%$

The maintenance check-up is repeated if the CV is >5% and inaccuracy is >10%. If the problem persists, request for instrument check-up and calibration should be lodged for further action.

2.3.4. Management of documents and records

Documents are communicators of quality system. They cover three main components, i.e. the policies, the processes and the procedures. The policies are basically about 'what to do', processes tell us about 'how it happens' while procedures explain 'how to do it' [15]. These components are communicated through quality manuals, standard operating procedures, working instructions, external documents and job and personnel-related documents.

On the other hand, records are information produced from the laboratory. Among all are forms, charts, test worksheets, patient records and reports, and quality control performance data [16].

Herewith are the documents we have in our Urinary Iodine laboratory:

i. List of documents for laboratory personnel
   - **a.** Work norms for Urinary Iodine laboratory staff
   - **b.** Checklist for Urinary Iodine laboratory staff
   - **c.** Daily work list for Urinary Iodine laboratory staff
   - **d.** List for work order, responsibility and relationships for Urinary Iodine laboratory staff
   - **e.** Annual work target for Urinary Iodine laboratory staff
   - **f.** Job description for Urinary Iodine laboratory staff
   - **g.** Summary of job responsibilities for Urinary Iodine laboratory staff

ii. List of documents for internal quality control
   - **a.** Levey-Jennings chart for Low control
   - **b.** Levey-Jennings chart for Medium control
   - **c.** Levey-Jennings chart for High control
   - **d.** Worksheet for IQC ranges determination

iii. List of forms
   - **a.** Test request form
   - **b.** Sample rejection form
   - **c.** Test report form
   - **d.** Worksheet for Urinary Iodine testing
   - **e.** Non-conformance and corrective action form

iv. List of instrument maintenance record forms
   - **a.** Heating block maintenance record form
   - **b.** Microplate reader maintenance record form
   - **c.** Micropipette maintenance record form

v. List of other related documents

- **a.** List of chemicals used for Urinary Iodine testing
- **b.** List of SOP, WI and external documents
- **c.** Procedural flow chart for Urinary Iodine testing
- **d.** Procedural steps for preparation of Urinary Iodine standards
- **e.** Main list of Urinary Iodine quality records
- **f.** Urinary Iodine standard preparation diagram
- **g.** Sample receipt record book
- **h.** Record book for results release through telephone

vi. List of standard operating procedures (SOP)

- **a.** SOP for Urinary Iodine testing
- **b.** SOP for maintenance of heating block
- **c.** SOP for maintenance of microplate reader
- **d.** SOP for maintenance of micropipette
- **e.** SOP for method validation
- **f.** SOP for preparation of IQC samples

vii. List of working instructions (WI)

- **a.** WI for sample collection, storage and transportation
- **b.** WI for sample rejection
- **c.** WI for sample disposal
- **d.** WI for glassware cleaning

Herewith are the records we have in our Urinary Iodine laboratory:

i. Pre-analytical stage

- **a.** Sample receipt (Test request forms)
- **b.** Sample rejection forms
- **c.** Instrument maintenance reports
- **d.** Internal quality control value determination reports

ii. Analytical stage

- **a.** Test worksheets

b. Instrument print-outs

c. Test results

d. IQC performance reports (Levey-Jennings chart)

iii. Post-analytical stage

a. Test reports

b. External quality assurance/proficiency testing reports

c. Non-conformance reports (NCR)

d. Management review meeting (MRM) minutes

In management review meeting, the performance of the laboratory is discussed. Source of problems is identified and corrective actions suggestions from staff are noted for further actions.

3. Establishing internal quality control

3.1. Preparation of in-house internal quality control (IQC)

IQC samples are used to verify the validity of laboratory results. Correct results for IQC obtained in an assay give the confidence that the patients' results are correct. The IQC samples are assayed as part of the analysis, together with the standards and patients' samples. The matrix of the IQC samples should be the same as the patients' samples; in the case of urinary iodine, the matrix is human urine. We use the pooled patient samples since there is no commercial IQC for urinary iodine yet in the market.

Pooled patient samples are usually mixed, aliquoted and kept frozen until use. The advantages of pooled patient samples are:

- The material is inexpensive since they are usually leftovers from the previous assays
- The determination of the concentration ranges is flexible since it can be adjusted accordingly
- Same matrix as human sample

The disadvantages are:

- The preparation of the IQC is time consuming
- The IQC materials can be infectious since there is no screening prior to pooling
- They are often unreliable since there are no preservatives added as stabilizers and their shelf life is often short (around 6 months)

Every time we prepare a new batch of IQC, the same procedures ought to be followed:

i. Analyze material for at least 20 runs

ii. Calculate the mean

iii. Calculate the standard deviation (SD)

iv. Determine range (mean ± 2SD)

The records of the IQC concentration range determination ought to be kept and referred to every time after assay. The IQC values obtained in an assay are compared to the mean ± 2SD values. Then, the IQC plotter charts are drawn (Levey-Jennings chart). Example is as depicted in **Figure 7**. The results should be checked; if the IQC values are within the ranges, the respondents' results are considered acceptable and could be reported. If the IQC values are out of range, the respondents' results are unacceptable for reporting and analytical problems need to be identified and solved. Daily IQC performance is very important in laboratories. It is very crucial to use fresh IQC samples in every assay. The IQC samples should be treated the same as treating the respondent samples.

3.2. Procedural steps in IQC preparation

1. Urinary iodine value of each respondent's urine sample is determined.

2. Urinary samples with the value within the target range are pooled:

- Low pool (L): 30–90 μg/l (e.g. target to get mean around 60 μg/l)
- Medium pool (M): 110–130 μg/l (e.g. target to get mean around 120 μg/l)
- High pool (H): 200–300 μg/l (e.g. target to get mean around 250 μg/l)

3. Urine iodine value of each urine pool is determined.

4. The target values are achieved using mixture of L pool and H pool through formulas 1 and 2 in **Table 2**:

5. Urinary iodine value of each modified urine pools is determined again and if the values are around the target values, the IQC pools are accepted as the new batch of IQC.

6. Urinary iodine values for each IQC are determined for at least 20 times (e.g. duplicates in 10 different assays).

7. After outliers are omitted, mean, SD and range (mean ± 2SD) are calculated for each L, M and H pools and these ranges are used to determine the validity of test results.

8. Every time after thawing frozen pooled urine, it ought to be centrifuged for 1000 g for 15 minutes, supernatant is then taken and mixed well. Pipette aliquots of 250 μl in 500 μl microcentrifuge tubes and keep at −20°C until use.

9. The IQCs in microcentrifuge tubes are thawed and are transferred into test tubes prior to assay, to be added with ammonium persulfate solution and ready for digestion with blanks and respondent samples.

10. Spike of urine samples with potassium iodate ought to be avoided since it is more unstable as compared to using the endogenous iodine in the urine matrix.

3.3. Monitoring QC performance through Levey-Jennings chart

QC results should be checked every time after an assay. There are some rules to refer to when deciding to accept an assay:

Accept assay when QCs are within 2 SD

Reject assay when any QC exceeds ±3SD

Reject assay when 2 consecutive QCs exceed ±2 to 3 SD

Reject assay when difference between 2 QCs exceeds ±4 SD

QC performance should be reviewed regularly to check the precision and accuracy of the assay. Both the acceptable and unacceptable results should be recorded. Corrective actions taken when QC results are unacceptable should also be recorded. Example of Levey-Jennings chart is as depicted in **Figure 7**.

Example:

Target volume = V_3 = 250 μl × 500 aliquots = 125,000 μl = 125 ml

Initial H pool concentration = M_1 = 400 μg/l

Initial L pool concentration = M_2 = 70 μg/l

Target concentration = M_3 = 250 μg/l

Volume L pool to be added = V_1 = X

Volume H pool to be added = V_2 = Y

Target volume = V_3 = 125

Formula 1: X + Y = 125; Y = 125 − X
Formula 2: $M_1V_1 + M_2V_2 = M_3V_3$; $M_1X + M_2Y = M_3V_3$

$M_1(X) + M_2(Y) = M_3(125)$
$M_1(X) + M_2(125 - X) = M_3(125)$
$M_1(X) + M_2(125) - M_2(X) = M_3(125)$
$M_1(X) - M_2(X) = M_3(125) - M_2(125)$
$(X)(M_1 - M2) = 125(M_3 - M_2)$
$X = \frac{125(M_3 - M_2)}{(M_1 - M_2)}$
$X = \frac{125(250-70)}{(400-70)}$
$= \frac{125(180)}{330}$
$= \frac{22,500}{330}$
= 68.18 ml
Y = 125 − X
= 125 − 68.18
= 56.82 ml

Table 2. Calculation to obtain the target values of control samples.

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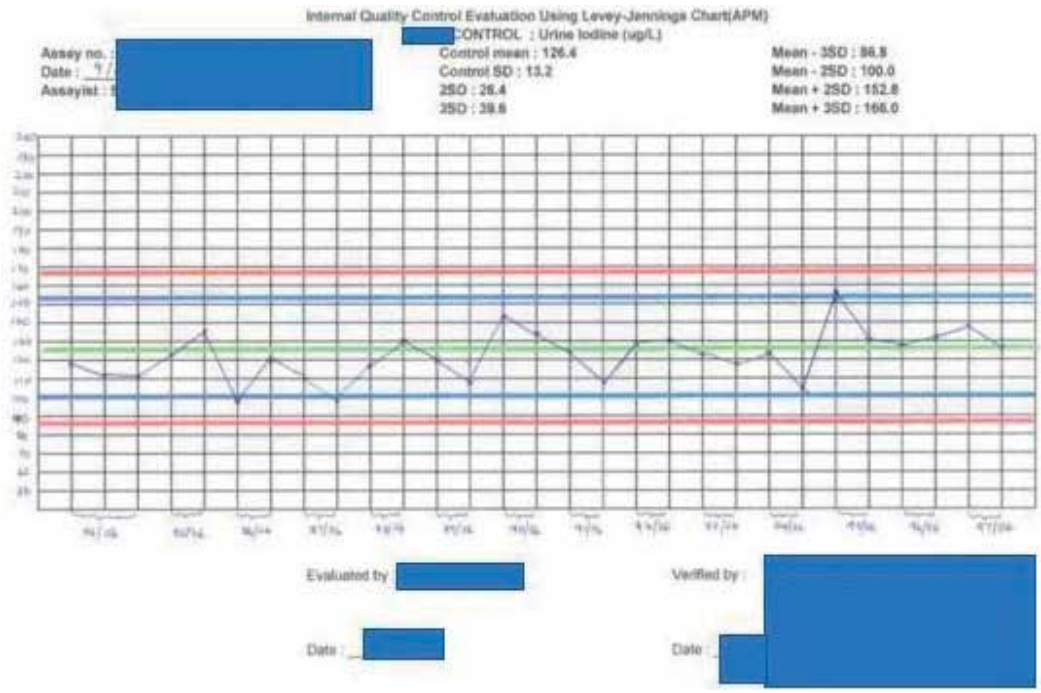

Figure 7. Example of Levey-Jennings chart to monitor IQC performance.

3.4. Non-conformance troubleshooting

If the mean value of the IQC samples is outside the range, the results for respondents in the same range cannot be reported yet and testing should be repeated. The QC charts (Levey-Jennings charts) trends should be checked and the drift in accuracy should be monitored. The cause of drifts should be investigated, e.g. faulty instruments (may cause systematic errors), expired reagents or IQC samples (may cause systematic errors), unclean glassware (may cause random errors) or changes in the laboratory environment (any contamination from iodized salt or elevated temperature may cause systematic errors), or human error (e.g. new operator assigned for the test may cause random errors). The investigation outcome is then comprehended among laboratory personnel and relevant trouble-shooting is taken. The trouble-shooting is recorded as corrective action and it is not a one-time solution to the current problem but also as a preventive step from the problem to occur again in future.

4. Participation in External Quality Assurance program

External Quality Assurance (EQA) is an ISO requirement, to confirm the quality of analysis. It shows the bias and precision of our assay and the position of our laboratory within the same

test method group and against other test method groups. Participation in the EQA program increases confidence of laboratory personnel in performing the analysis.

There are various methods which are under the External Quality Assurance Program, i.e. Sandell-Kolthoff method consists of three different assays: (1) done in tubes, digestion with chloric acid; (2) done in tubes, digestion with ammonium persulfate; (3) done in microtiter plate, digestion with ammonium persulfate. There are other methods such as using the autoanalyzer using dry ashing of urine in potassium carbonate. However, the method with highest sensitivity is the inductively-coupled plasma-mass-spectrometry (ICPMS) but small/medium scale laboratories may not afford to purchase the instrument.

CDC's Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program from the Centre for Disease Control and Prevention (CDC), Atlanta, U. S. A. is worldwide. To date, more than 84 iodine laboratories from more than 50 countries have participated. Our laboratory has participated since the year 2010 until present.

If any urinary iodine laboratories are interested to participate in the EQA program, please visit CDC's website. Application form should be completed and e-mail or fax it to CDC, and a confirmation e-mail will be received within 72 hours. The laboratory will then be enrolled immediately upon receipt of the form and will receive a set of EQA samples every February, June and October each year.

What each laboratory should do is to treat the EQA samples like normal respondent samples and run the EQA samples in duplicates in three assays on different days. Report of the results should be submitted before the deadline within 1 month after receipt of samples.

5. Evaluation of performance of urinary iodine laboratories

Throughout a time-frame, there is need for an evaluation to be done on our urinary iodine laboratory performance. The UIMM had been validated in the year 2006. Since then, all Urinary Iodine laboratories in the country had started using the method. In the year 2008, the National IDD Survey had been carried out [17] and the urinary iodine assays was performed on the respondent samples with inclusion of IQC samples and EQA samples in every assay. The EQA samples were provided by the Institute for Clinical Pathology & Medical Research (ICPMR), Australia. Thus, with the available data, an evaluation on our laboratory performance was performed using the EQA sample results by applying the Six Sigma quality metrics [10]. The higher the sigma metrics the better, and Sigma-6 is the best, depicting very little error or errorless achievements. A method decision chart [11] was plotted for all laboratories (it was four laboratories at that time) and the achievement for every Low, Medium and High controls were determined. The method validation results were also plotted on the chart. There were two plots, i.e. one is set by The Urinary Iodine Quality Program (TUIQP) under ICPMR (**Figure 8**) and the other one is following the limit set by Ensuring Quality in Urinary Iodine Program (EQUIP) (**Figure 9**) under CDC. However, the latter EQA program had been halted

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and the former EQA program is the only existing program providing services to various urinary iodine laboratories worldwide. Since urinary iodine is easily evaporated and UIMM uses digested sample of only 30 μl out of 1250 μl of total volume, high %CV is usually high in the lower concentration range of the standard curve. EQUIP which set stricter %CV limit, leads

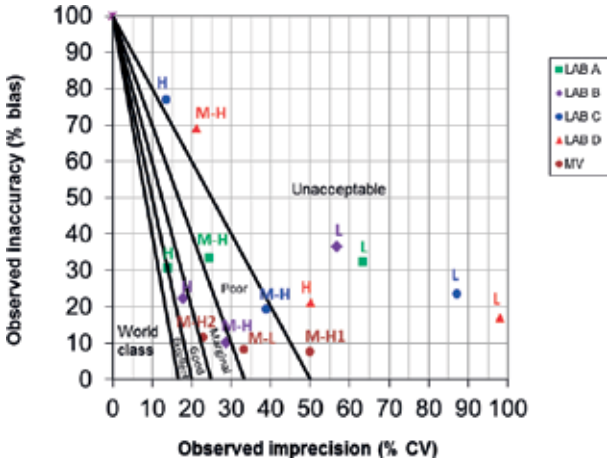

Figure 8. Urinary iodine micromethod's normalized method decision chart against The Urinary Iodine Quality Program (TUIQP) TEas based on the 2008 National IDD Survey EQA results (Lab A [n = 20], Lab B [n = 18], Lab C [n = 12], and Lab D [n = 6]) and the 2006 method validation study (MV). L indicates low control, M-L indicates medium-low control, M-H indicates medium-high control, and H indicates high control, according to urinary iodine ranges in **Table 1** of reference [10] as reported in Hussain et al. [10] (image reproduced with permission of the rights holder, Annals of Laboratory Medicine).

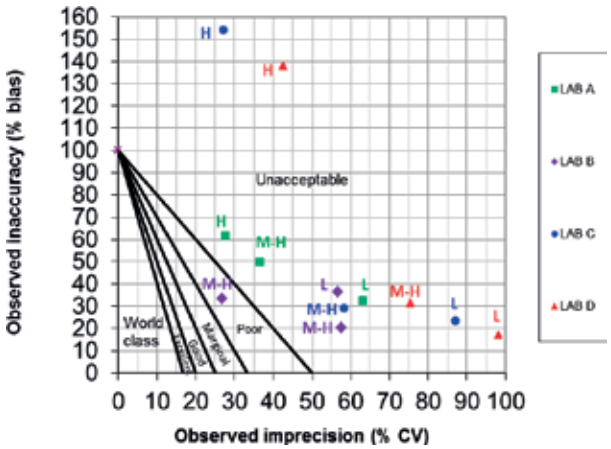

Figure 9. Urinary iodine micromethod's normalized method decision chart based on the 2008 National IDD Survey EQA results (Lab A [n = 20], Lab B [n = 18], Lab C [n = 12], and Lab D [n = 6]) and against EQUIP TEas. L indicates low control, M-H indicates medium-high control, and H indicates high control, according to urinary iodine ranges in **Table 1** of reference [10] as reported in Hussain et al. [10] (image reproduced with permission of the rights holder, Annals of Laboratory Medicine).

to lower sigma metrics for the Low control (**Figure 9**). High control is easier to pass the %CV limit set by both EQA providers (**Figures 8** and **9**).

Before participating in the EQA program, another way to evaluate the nation's urinary iodine laboratories was through inter-laboratory comparison. Some analyzed respondent samples were chosen from a wide range of urinary iodine concentrations and the same samples were analyzed again by another urinary iodine laboratory by using the same method. The results were compared between the laboratories and biases were determined. Any discrepancies were then discussed and trouble-shooting to problems was carried out.

6. Safety and waste management

Safety and waste management is very important and included in the quality management system. All Material Safety Data Sheets (MSDS) for every chemical used were printed out and kept in a designated file for reference. The information is used for self-awareness and protection, waste labeling and in any spillage incidences. Wearing proper personal protective equipment (PPE) should be a culture in the urinary iodine laboratory.

6.1. Safety

One of the chemical used in the UIMM is arsenic (III) oxide. It is categorized as highly toxic. Thus, safety precautions ought to be made along the way from purchasing, storage, analytical stages until waste disposal. The safe procedure to weigh arsenic is as discussed below as reference to all operators. Its storage should be in a locked containment with records of its date and time it is being taken out and name of the operators handling it.

6.1.1. Know your urinary iodine chemicals

The properties of UIMM chemicals and precautions that should be taken while handling them are as stated in **Table 3**.

Chemical (properties)	Precaution
Ammonium persulfate (oxidizing substance)	Avoid direct contact to skin or inhalation
Ceric ammonium sulfate (toxic substance)	Avoid direct contact to skin or inhalation
Arsenious acid solution (highly toxic substance) (containing sodium chloride, sodium hydroxide, arsenic (III) oxide and sulfuric acid)	Avoid direct contact to skin or inhalation
Potassium iodate (toxic substance)	The concentration of the working solution is not exceeding hazardous limit but precautions while handling it ought to be taken
H_2SO_4 (corrosive substance)	Avoid direct contact to skin or inhalation

Table 3. Chemical properties and precautions when handling UIMM chemicals.

6.1.2. Weighing of arsenic

1. It is a must to wear R95 or N95 mask while weighing arsenic substances as it is highly toxic and can affect pulmonary system if inhaled accidentally.
2. Prepare two 100 ml beakers:
   a. one is empty
   b. one is filled with 20 ml dH_20
3. Weigh the chemical in the empty beaker
4. Take out the beaker from the weighing scale
5. Pour the water from the second beaker into the first beaker by letting the water flow slowly into the first beaker. This will avoid the chemical from floating into air when transferring the beaker to the work bench.

6.1.3. Other safety precautions

- Do not drain the reagents in the sinks
- Arsenic is highly toxic. Limit of arsenic that can be drained through the laboratory sinks with permit is 0.003 mg/l [18]. Thus, all urinary iodine assay waste should be poured into appropriate waste containers before the glassware is soaked and washed
- Send the waste for disposal properly as discussed in Section 6.2.

6.2. Waste management

- Labels on the waste containers should be legible and clear
- Name of waste and category of waste should be written and printed on the label
- Date of the first time the waste is accumulated and date of the last time the waste is accumulated should be written on the waste label
- A void space in the waste container of approximately 10% should be allowed for expansion
- Waste should be stored in closed containers, placed in a corrosive-proof basin as secondary containment against spillage
- Waste containers for urinary iodine should be placed in the same room but not mixed with wastes from other analysis
- Each reagent waste ought to be placed in individual waste bottle, labeled and dated
- Avoid from putting unbalanced reaction mixture in one waste bottle; this may lead to accumulation of gas and the waste bottle may explode
- Aware of toxicity of reagents.

7. Way forward

7.1. Training

Training of staff running the urinary iodine assays ought to have these goals:

- Achieve competency to do laboratory work
- Understand aspects in the laboratory Quality Plan
- Aware of sensitiveness of urinary iodine test
- Aware of possibility of contamination from iodized salt
- Implement correct waste handling system
- Abide by reagent expiry dates.

7.2. Way forward for small/medium-scaled urinary iodine laboratories

The current reference method for urinary iodine testing is the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). However, this instrument is most likely unaffordable by small/medium-scaled laboratories due to its high purchasing and maintenance costs. Even though there is high tendency of getting higher bias and deviation when using Sandell-Kolthoff method as compared to using ICP-MS, the small/medium laboratories can still obtain excellent performance by applying a closely-monitored quality management system in the laboratories as discussed in previous sections in this chapter. Decision to participate in the International Organization for Standardization (ISO) 15189 for Quality and Competence for Medical Laboratory will be a plus-point since it consists of all the elements of quality management and ensures quality in the results produced by the laboratory.

8. Conclusion

Even though UIMM, a spectrophotometrical method, is less sensitive compared to the sophisticated methods such as the ICP-MS, the same process of quality system applies to the latter as well. Since urinary iodine is easily evaporated, careful measures have to be made in all pre-analytical and analytical procedures to minimize it. The quality system is supported by detailed documentation and glassware cleaning in the post-analytical procedures. IQC and EQA programs are very important to enhance validity of respondent results to be released to the IDD program managers in monitoring the population iodine status. Urinary iodine estimation is vital to maintain effectiveness of the USI program in eliminating IDD worldwide. This chapter is hoped to be a guide to all urinary iodine laboratories in understanding quality control in urinary iodine estimation.

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Conflict of interest

It is declared that there is no conflict of interest involved in publication of this book chapter.

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Chapter 7

Errors in Surgical Pathology Laboratory

Monique Freire Santana and
Luiz Carlos de Lima Ferreira

Additional information is available at the end of the chapter

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Abstract

Pathology must aim at a correct and complete diagnosis for the patient, which is timely, useful, and understandable to the physician assistant. However, in daily practice, there are multiple possibilities of errors in the pathology laboratory, with several impacts on patient care and prognosis. In this chapter, we discuss the different concepts of error and diagnostic concordances in pathology, at which point in the diagnostic process the errors are more frequent, and propose solutions to minimize the chance of their occurrence.

Keywords: medical errors, surgical pathology, pathology errors

1. Introduction

In 1999, the American Institute of Medicine (now the National Academy of Medicine) published the paper "To Err is Human: Building a Safer Health System" [1], which broadly defines medical error as the inability to complete a planned action or the use of a wrong plan to achieve a goal. Sirota summarizes the document and its implications for pathology. In his chapter, the author considers that the efforts of professional societies, such as the College of American Pathologists (CAP), through the Laboratory Accreditation Program, as well as their councils and commissions, determine the quality standards for the practice of pathology. In professional training, the academic programs and the American Board of Pathology, with their certification mechanism, help to ensure the full competence of the practice of pathology [2].

The year 1989 saw the most famous quality control initiative when the CAP introduced the Q-PROBES Program, which defines quality in terms of practices of laboratory medicine and anatomic pathology. At the same time, 118 Q-PROBES studies have been conducted in

thousands of hospitals and independent laboratories in the USA, other places from North America, and abroad to identify and describe various experiences. These studies investigate the frequency of errors occurrence: the laboratory participants submit data from the calculation of the normative rates of errors during the laboratory tests. This exchange of information occurs so this knowledge convinces laboratory to abandon practices and behaviors harmful in the process of laboratory tests [3].

Some reasons may explain less attention to errors in medical labs when compared to other medical errors. The higher variability in error during laboratory testing, difficulties in screening all errors, and all steps involved in the total testing process (TTP) can help explain these facts. Besides that, the TTP is more complex and needs cooperation between several health institutions. Surprisingly, physicians and other interested people do not understand full aspects about the harmfulness of errors in laboratory medicine. In addition to that, it is undesirable for laboratory professionals to report and disclose data about errors [4].

The errors in pathology laboratory are so common that in a self-administered mailed survey with 260 practicing pathologists and 81 academic hospital laboratory medical directors, approximately 95% suggested the involvement of any error and only 48% of that professionals believed that current error reporting systems were adequate. Among the factors that might make it less likely that they would disclose a serious error to a patient, the most common was the possibility that the patient would not understand what he or she was being told (n = 84, 49.7%) and the physician would not be able to explain the error clearly to the patient (n = 68, 40.2%), according to the interviewees' perception. The majority of participants believed that minor and near misses needed to be disclosed to patients (n = 120, 72.3% and 34, 20.1%, respectively) [5].

Troxel stands that an expectation from the society of "zero diagnostic error" and the "zero error standard" supported by the US judicial system is unattainable for obvious reasons (6). Surgical pathology laboratory process is much more complex than highly mechanized processes with minimal human participation, such as clinical laboratory analysis. Meier [6] describes the pathology production process in 12 steps. The production process begins with the correct identification of the patients' samples (1), selecting tissue specimens (2), labeling and transport (3), and accession (4). The process continues with the description of steps involving receipt and sampling of specimens (5), fixing, embedding, and cutting section (6), mounting, staining the slides, and labeling them (7), and delivering them to surgical pathologists (8). The process continues at the pathologist's desk—with examining, collating, and interpreting slides (9) and examining the possibilities of ancillary tests or other information (10), the composition of reports, (11) and finally the receipt and interpretation of the report (12). Therefore, the surgical pathology report is a complex task with multiple steps in which there is a possibility of error.

Meier et al. [7] proposed a standardized error classification that until then did not exist in pathology. We describe four types of errors (defective identification, defective specimen, defective interpretation, and defective report), distributed according to the processing step in the laboratory. In the pre-analytic phase, they describe defective identification (patient, tissue, laterality, anatomic location) and defective specimen (loss of the specimen, erroneous in measurement or gross description, floaters, inadequate sampling, and the absence of

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indication of ancillary studies when necessary). The analytic phase includes errors in classification, false negative or positives, and in post-analytic phase, and they describe the defective reports (erroneous or missing nondiagnostic information, error in dictation or typing, report delivery and errors related to computer or format, transmission and upload error). During the pre-analytic phase, wrong identification can be responsible for 27–38% of the errors, and specimens-related errors vary from 4 to 10%. In the analytic phase, diagnoses misinterpretation occurs from 23 to 28% of the errors, and in post-analytic phase, the defective report included from 28 to 48% of the errors. This proposed error taxonomy has shown a very good interobserver agreement of 91.4% ($k = 0.8780$; 95% confidence limit, 0.8416–0.9144) when applied to amended reports.

2. Diagnostic errors and concordances in pathology

To discuss the errors in pathology, it is essential to conceptualize their goals. Pathology should provide a correct and complete diagnosis, in other words timely, useful, and understandable for the attending physician [8]. Since the goals of pathology are multifaceted, it is easy to understand that there are multiple possibilities for error. The result must be accurate, based on gold standards, and scientifically validated. But what is the gold standard of pathology? Morphology is subjective and affected by the observer's experience. Cytogenetic studies by in situ or molecular hybridization are not applicable to most diseases routinely found in surgical pathology. Therefore, the most appropriate is to determine the accuracy, as a measure of diagnostic adequacy; it suggests that most of the qualified pathologists will agree on a similar diagnosis when analyzing the same specimen. A major or unacceptable variation is the one that will have a great effect on therapy or prognosis, such as in classifying a benign tumor as a malignant one. A smaller, acceptable, or minor variation is the one that has no effect on the treatment that would alter the progression of the disease, with no effect on the prognosis, such as in some subclassifications of benign or malignant tumors. These definitions can be applied to the three pathology goals (correct, complete, timely) [8, 9]. The errors can be further divided into errors of accuracy, that is, how much of the released diagnosis represents the true pathological process and precision errors related to concordances among pathologists in the interpretation of a case [9].

Meier et al. [10] divided the errors of pathological reports into four categories: errors of interpretation, identification, the specimen, and related to the report. A study based on this classification evaluated 73 participating institutions of Q-PROBES with 1688 errors in 360,218 cases of surgical pathology, with a ratio of 4.7 errors/1000 cases. Rates were higher in institutions with pathology residency programs (8.5 vs. 5.0/1000, $p = 0.01$) or when a percentage of cases were reviewed after release (6.7 vs. 3.8/1000, $p = 0.10$). Interpretation errors were responsible for 14.6% of the cases, 13.3% were identification errors, 13.7% were related to specimen errors, and 58.4% errors were of other modalities. In general, more errors were detected by pathologists (47.4%) than by clinicians (22%). Incorrect interpretations and specimen errors were detected by pathologists (73.5% and 82.7%, respectively, with $p = 0.001$), while identification errors were more frequently detected by other physicians (44.6%, $p = 0.001$). The rates of identification errors were lower when the reports were reviewed by a second pathologist

prior to their release (0.0 vs. 0.6/1000, $p < 0.001$), and errors related to the specimen were less reported when released after an intradepartmental review of more difficult cases (0.0 vs. 0.4/1000, $p = 0.02$) [11].

Meier [6] describes why the comparison of discrepancy rates is difficult in six different steps between the initial diagnostic event and the review event. The first is the difference in the internal and external review. In the internal review, the diagnoses under scrutiny were originally performed in the laboratory, and pathologists in other practices performed an external review. Second, the pre-sign-out review was held before a report was issued. Post-sign-out review happened after the report had been released. Third, in conference reviews, several experts discussed information about diagnosis, prognosis, and treatment of the patient to reach an agreement. Some reviews were nonconference related. Fourth, in review performed by an expert, the exam was conducted by a specialist with extensive experience and knowledge in the field. The fifth difference was blinded and nonblinded reviews. In blinded revisions, the second pathologist had the same amount of information as the first one, and sometimes a blinded reviewer was given less case-specific information. The last difference was between focused reviews in which the examiner trained the diagnosis of specific types of cases and nonfocused revisions in which the pathologist evaluated a defined fraction of cases of various specimens or types of diagnoses.

3. Where is the possibility of error?

Valenstein and Sirota [12] described four classifications of errors:

1. Depending on the scenario in which error occurred, in pre-laboratory errors (identification errors external to reference laboratory) and laboratory errors. The second form of this classification is the division of errors into pre-analytic, analytic, and post-analytic errors. This is the most common classification of errors, based on the time and place of the laboratory where they occurred: in the pre-analytical, analytical, and post-analytical phases. This division is commonly used in clinical analysis laboratories and, since they are based on similar work processes, they may be used to evaluate work in pathology.
2. Consequences for the patient: in this case, the errors are distributed in near misses (or "close calls"), when the error is detected before causing harm to the patient. On the other hand, adverse events damage the health of a patient, such as a new biopsy or unnecessary procedure. Sentinel event is serious, which may cause permanent disability or death because of errors.
3. Type of error: patient misidentification or specimen misidentification.
4. Cause of error: based on the root cause of identification errors—human factors, environment, equipment failure, and lastly defective rules, policies, or procedures [12].

In a study to develop a reproducible amendment taxonomy, Meier et al. [13] described a classification in four categories: misinterpretations, misidentifications, defective specimens, and defective reports.

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1. Misinterpretations: This category is divided into three subtypes that occurred in relation to two levels of diagnostic information. In the first subtype, the diagnostic conclusions described inaccurate information (false-positives or overcalls). In the second subtype, the pathologist failed to recognize or lost accurate information (false-negatives or undercalls). Both can occur at primary (such as changes between positive and negative status or between malignant and benign diagnosis) and secondary levels of diagnosis. The secondary-level diagnosis refers to when the clinical context or prognostic implications depend on the pathologic diagnosis, which occurs in malignant tumors.

   The third subtype is misclassifications that occur when the pathologist changes similar diagnostic categories, for example, the names of a soft tissue sarcoma, without primary diagnostic implications or secondary diagnostic information's modifying impact (the differently labeled sarcoma behaved biologically with the same degree of aggressiveness during the same treatment).

2. Misidentifications: contained four subtypes—patient identification (lacking or wrong); tissue designation (e.g., lung confused with liver); laterality specification; and anatomic localization (e.g., skin of head misidentified as skin of hand).

3. Specimen defects included five subtypes: lost specimens, specimens with inadequate sample volume or size, samples with absent or discrepant measurements, inadequately representative sampling, and samples with absent or inappropriate ancillary studies.

4. Report defects: Defects of three subtypes were observed. In the first subtype, missing or erroneous non-diagnostic information about practitioners involved in the case, procedure or dates in which the specimen was collected, or codes regarding the patient, procedure, or diagnosis, and so on. The second subtype may be dictated or typographical errors. Failure or aberrations in electronic formats or in the transmission of information in reports was considered the third subtype of error.

During the material reception, gross examination, and processing, there are many possibilities of error, from the exchange of samples or labels, absence or excessive cuts in the block, to cross-contamination with tissues foreign to the specimen included in the final slide. Cognitive errors, such as inadequate or incomplete macroscopic descriptions, inadequate representation of the lesion or of relevant areas necessary for its characterization, may also occur, and although some are beyond the pathologist's control, the responsibility falls directly on him, with very serious damage to the patient [8].

Morelli et al. [14] described critical points in pre-analytical steps in a pathology laboratory of a leading hospital in Lombardy, Italy. In this work, 8346 histological cases were reviewed, for which 19,774 samples were made and from which 29,956 histologies were prepared. They identified 132 errors, such as accessioning (6.5%), gross dissecting (28%), processing (1.5%), embedding (4.5%), tissue cutting and slide mounting (23%), coloring, (1.5%), labeling, and releasing (35%). Some very common errors were not detected in this work: specimen mismatching and sample contamination in gross room; mismatching or loss of specimen in embedding, loss, exhaustion, or contamination of specimen; and damage or changes of samples on the slides

in cutting and slide mounting. As expected, 98.5% of the errors were due to a lack of attention, and the majority had no consequences for the patient (88%). Only 10% of the errors resulted in a delayed report to the physician. Overall, 85% of errors were detected during gross dissecting, tissue cutting or slide mounting, labeling, and releasing, and 80% of errors could be due to incorrect transcriptions of container identification, on slides, and on labels applied to the slides at the time of delivery. The quality of the slides is a prime factor for the correct diagnosis. In some cases, problems in the cutting, staining, or assembling of the slides can completely prevent an adequate diagnosis (**Figures 1** and **2**).

A study carried out in Pennsylvania, in a teaching hospital with Pathology residency training, identified 491 errors. Of these, 88% (n = 432) of errors were found in the pre-analytical phase, in terms of the order, identification, collection, transportation, material reception, and processing in the laboratory. The authors identified 20% (n = 4) of analytical and 39% of (n = 8) post-analytical errors [15], as shown in **Table 1**, associated with Tosuner [16] survey data.

It is important to emphasize that the risk of loss or exchange of the specimen is critical in the pre-laboratory stage, from the moment of its collection, registration, gross description, and confection of the slide. Morelli et al. [14] described additionally in pre-laboratory phase: the presence of extraneous tissue (ET), mistaken specimen, excessive number of containers in gross dissecting, the absence of decalcification of the specimen when necessary, loss or exhaustion of specimen in tissue cutting, wrong choice for thickness section, error in identification of block to be cut, and others.

Some pre-analytical artifacts are caused by improper manipulation during the biopsy procedure. Excessive tissue trauma caused by tweezers and other surgical instruments (**Figures 3** and **4**), as well as the excessive use of electrocautery in the surgical margins, provoke artifacts that may lead to the need for a new biopsy collection.

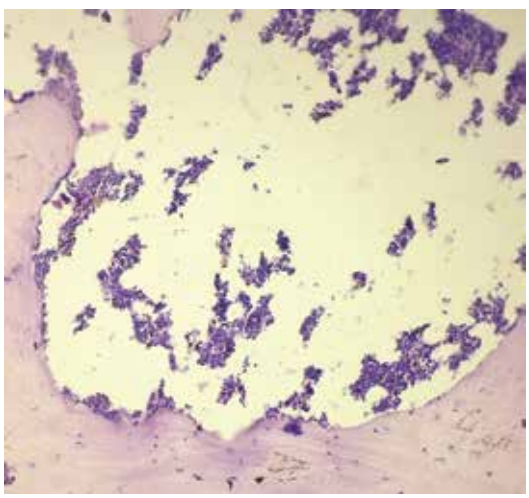

Figure 1. The inappropriate cut makes it impossible to evaluate the cellularity of this biopsy (Bone marrow, H&E, 400x).

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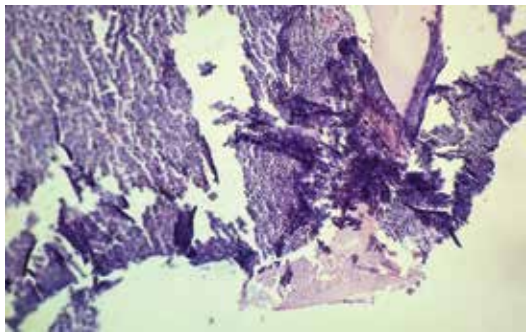

Figure 2. The presence of folding in tissue does not allow adequate observation of the morphological characteristics (Bone marrow, H&E, 400x).

Layfield and Anderson [17] evaluated the sample labeling errors in 29,479 cases associated with 109,354 blocks and 248,013 slides for 18 months. In identification errors, a sample was labeled with the incorrect name or identification number. In the case of samples pertaining to identification errors, a specimen was incorrectly identified as to the site of origin at the time of collection. The authors identified 75 errors; of which 55 (73%) were related to the patient's name and 18 (24%), to the anatomical site. Most of the mistakes (69%, $n = 52$) occurred in the gross examination room, 19 (25%) in the histology laboratory, and four (6%) were related to the pathologist's errors. From the errors, 73% ($n = 55$) resulted in slides assigned to noncorresponding patients. Most of the identification errors occurred in skin, esophagus, kidneys, and colon biopsies, reflecting the distribution of types of cases received in surgical pathology, with small samples from endoscopy and dermatology.

Bixenstine et al. [18] observed 69 hospitals in 3 months and described identification defects in 2.9% of cases (1780/60,501; 95% confidence interval [CI] = 2.0–4.4%), 1.2% of containers (1018/81,656, 95% CI = 0.8–2.0%), and 2.3% of requisitions (1417/61,245, 95% CI = 1.2–4.6%). In container defects, the authors included missing specimen, container with no identified or misplaced label, absence or incorrect numeric patient identifier, absence of specimen type or source, and/or incorrect specimen type or source (or laterality). Requisition defects included the absence of requisition (or a blank requisition), date, time, name, specimen source/type, laterality, and/or numeric identifier (or when this information was wrong).

We routinely observe the widespread use of inadequate containers, too small for the specimen, which make it difficult to withdraw. It is recommended that containers can be used to allow the material to flow without deformities. Some deformities are caused by the narrow fit of the part in the container, which prevents its proper fixation. In addition, the bottle should contain 10–20 times the volume of the piece in a fixative solution and the specimen.

In the cases of small biopsy, the risk of change in gross pathology is more dangerous. Sometimes histology shows evidence of suspicious exogenous tissue sample, such as tumor cells with nuclear inclusions similar to arachnoidal cells in an endometrial sample, associated with the presence of eosinophilic amorphous material morphologically similar to secretory meningioma. Some techniques can be helpful to identify mixed-up tissue specimens, such as microsatellite PCR techniques and another [19, 20].

Preanalytical phase[1,2] : 53.3 [22] **to 88%** [21]
Deliver and registration of material
Incomplete/error in order
Order does not correspond to specimen
Sample quantity does not correspond to order
Specimen without previous marking/incorrect orientation
Incorrect anatomical site
Incomplete/inaccurate clinical information
No material in sample sent
Inappropriate packaging/fixing conditions
Specimen loss in laboratory
Integrity not preserved
Malfunction of equipment
Freezing error
Register error
Analytical phase: 4 [21] **to 42.1%** [22]
Quality of the slides
Repetition of coloration
Foreign tissue in the specimen
Incorrect block identification
Interpretation errors
Delayed results
Work environment (e.g., refrigeration failure and other equipment failures)
Postanalytical phase: 5.6 [21] **to 8%** [22]
Correlation errors of freezing biopsy with conventional histology
Specimen discarded during routine examination
Patients exchange
Transcription errors
Delayed results
Malfunction of laboratory information systems

[1]Preanalytical phase include accessioning, gross dissecting, processing, embedding, tissue cutting, mounting, coloring, labeling and releasing slides. Some errors outside of laboratory were included in this category for didactics effects, such as identification mislabeling, loss of specimen etc., because these errors may occur in or out of laboratory. Besides that, some errors (e.g., contamination or loss of specimen) can happen in several steps inside the laboratory, since gross dissecting, embedding or tissue cutting until slide mounting.

[2]Another preanalytical errors describe for Morelli et al. [20] include specimen wrongly accessioned, incorrect numbering of the blocks or slides, decalcification not performed or insufficient, error in procedure temperature, specimen badly positioned, number was reported incorrectly in block or slide, error in thickness selection and loss or exhaustion of specimen in cutting, wrong coloring (manually) or error in the choice of the program (in automatic coloring).

Table 1. Distribution of errors according to the operating process phase and examples.

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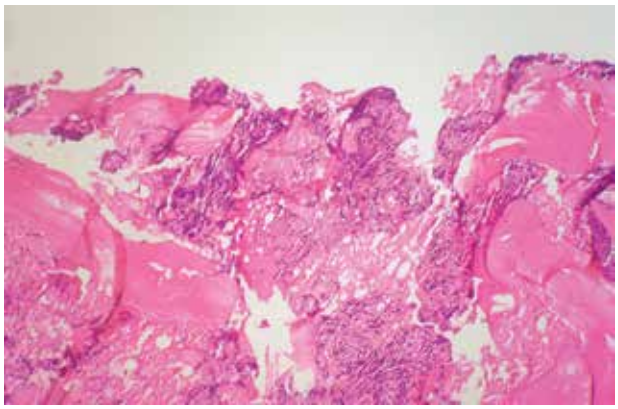

Figure 3. The excess of crushing at the time of biopsy collection makes it impossible to properly evaluate the cellular morphology in this bone marrow (Bone marrow, 100x, H&E).

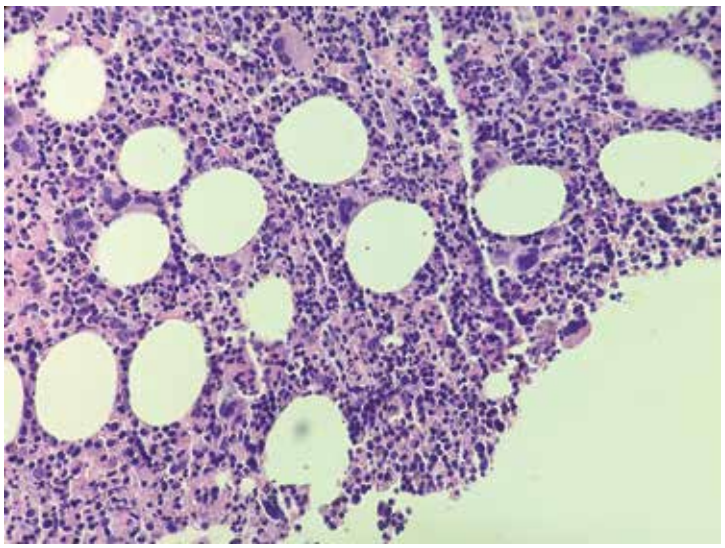

Figure 4. In contrast, in adequate sampling, it is possible to define the morphology of the cellular activity with perfection (Bone marrow, 400x, H&E).

In an accessioning, many errors can occur. For example, the use of Roman numerals for labeling sample bottles can lead to confusion when the numbers 3 and 4 (III and IV, respectively) cannot be distinguished clearly. In other cases, the extravasation of formalin or another fixation solution can clear the identification in the biopsy bottle. It becomes more critical when there are several biopsies of the same patient from different anatomic places. In some cases, only the precise information in the request form can make the pathologist think of a possible mix-up of species. Besides that, the identification in the laboratory is critical as well. Even when clearly written, the numbers for slide identification can lead to confusion, such as when the lower horizontal bar of the number 2 on the middle slide is rather short and can be mistaken as number 7 [21].

In gross macroscopic examination, cutting or staining of the slides, contaminants can be a rise, often called "floaters" by laboratory staff, and most of the time it is easily recognized as such. However, contamination of patient samples by strange tissues of a similar type may represent a higher risk for misinterpretation, as in the cases in which malignant tissue fragments are found in biopsies from patients without malignancy. Carpenter [22] described that the first opportunity for this error occurs during gross examination and dissection and that some specimen types that are considered high risk for cross-contamination: esophageal biopsies, endocervical curettage specimens, and lymph nodes biopsied for metastatic malignancy. For example, contamination of an esophageal biopsy by a very small fragment of normal tissue from the small intestine or colon may lead to a false-positive diagnosis of Barrett's esophagus or, worse, when the contamination occurs by a fragment of atypical or "dysplastic" intestinal epithelium that may lead to a false interpretation of Barrett's esophagus with "dysplasia." In these cases, the productivity of the entire laboratory decreases until the pathologist discovers the source of contamination because of the longer evaluation time and the need to deepen the histological sections. This risk is foremost in laboratories that specialize in one area of the anatomic pathology (e.g., dermatopathology, gastrointestinal pathology, etc.) because most of the specimens are of a similar type, making it difficult to recognize the floaters. In a laboratory where prostate biopsies are exclusively evaluated, a little fragment of the prostate is less likely to be identified as extraneous. To reduce this risk, it is essential that a gross station stay clean and organized.

The tissue floaters can be found in histology water baths and the slide stainers. In a study performed by Platt et al. [23], extraneous tissue found in stain bath, ranging in size from two to three cells to hundreds of cells, and the principal source of contamination was represented for the first sets of xylenes and alcohols. Of 13 water baths examined, only one fragment of tissue was identified.

In the largest study of extraneous tissue (ET) in surgical pathology, with data about 275 laboratories included in Q-Probes, the quality program of CAP describes the frequency of ET in two steps: a prospective and retrospective slide review. An extraneous tissue rate of 0.6% of slides (2074/321757) in the retrospective study and 2.9% of slides (1653/57083) was detected. In 0.4 and 0.1% in the prospective and retrospective phase, respectively, the presence of ET caused difficulties in the diagnostic conclusion [24].

Deficiencies in pre-laboratory steps can occur as well. In a study with 417 laboratories in the College of American Pathologists' voluntary quality improvement program (Q-Probes) identification and accessioning deficiencies were found in 60,042 (6%) out of a total 1,004,115 cases accessioned (median deficiency rate of 3.4%). Identification of specimen was done incorrectly in 9.6, 77% errors in discrepant or missing information, and 3.6% involved specimen handling. Absence or incomplete clinical history or diagnosis on the requisition slip represented 40% of all deficiencies. A correction was done in 69% of cases involving specimen identification errors, 58% of correction was done in specimen handling errors, and 27% of cases with discrepant or missing information. Lower rates of deficiencies were identified in laboratories with lower numbers (<15,000) of accessioned cases and laboratories with a formal written plan for the detection of this type of errors [25].

Analytical errors generally have greater evidence of impact on patient care, with potentially devastating consequences for them and the responsible pathologist. Troxel [26] reviewed records of

lawsuits against pathologists for diagnostic negligence at a US insurance company responsible for the insurance of 1100 pathologists. The pathology presented a low frequency of complaints (8.3% per year) and, however, with a great financial impact, measured by a number of indemnities paid per claim since many claims against pathologists resulted from the lack of diagnosis. False-negative and false-positive results for cancer accounted for 63 and 22% of claims, respectively. The highest values were related to diagnostic errors in melanomas (US$757,146; 95% false negatives), cervicovaginal cytology (US$686,599; 98% false negatives) and breast cancers (US$203,192, with the same proportion of false negatives and positives). Also with respect to analytical errors, Genta [27] argued that there are external or "suprahistological" elements that interfere with the pathologist's decision which can be divided into two categories: the evidence-based ones (such as age, sex, ethnicity, and epidemiology) and the elements that arise from emotional perceptions, not rooted in objective evidence, named emotional elements, directly related to inter and intra-observer variability. Faced with a colon adenoma with high-grade dysplasia, the pathologist may believe that surgeons will interpret the presence of dysplasia as a license for an unnecessary surgical resection and feel inclined to omit such information from the report. Even the errors of pathologists, when discovered, may modify their decision-making behaviors. Biases such as visual anticipation, first impression, and preconceived judgments influence the critical decision-making processes [28]; however, to what extent such elements may interfere with the pathologist's diagnostic decision-making is uncertain.

It is known that it is strongly recommended that pathologic diagnosis has the following characteristics: (1) accuracy and precision of report, (2) completeness of report, and (3) timeliness. The accuracy is based on scientifically validated gold standards, and it can be difficult since most of the diagnoses do not have this pattern in morphological analysis. The pathologic diagnosis depended on interpretative and subjective skills. The precision is a measurement of variation, and a minimal interobserver variation is a major goal in pathology diagnosis [29]. In a review of 344 pathology claims reported to The Doctors Company from 1995 to 1997, Troxel identified 218 claims related to surgical pathology; of these, 54% represented claims in six groups of specimen type or "high-risk" diagnostic areas, which included breast biopsy, melanoma, lymphoma, fine-needle aspiration, frozen section, and prostate biopsy. False-negative diagnosis of malignancy represented 52% of these claims, and 33% of these were false-positive diagnosis [30].

In Pakistan, Ahmad et al. [31] performed a study to describe the frequency and types of error in surgical pathology reports. They found errors in 210 cases (0.37%) after analyzing 297 reports during the study conducted on 57,000 surgical pathology cases in a laboratory in Karachi in 2014. These comprised 199 formalin-fixed specimens and 11 frozen sections represented as 3.8% of a total of 2170 frozen sections. Of this—11 frozen section errors—10 were misinterpretations and the most comprised malignant diagnosis in the central nervous system. Of the 199 permanent specimens, 99 (49.7%) were misinterpretations, and the most common subspecialty/anatomic location was gastrointestinal tract (including liver, pancreas, and biliary tract) with 23.2% (n = 23), followed by breast (n = 13, 13.1%), and lungs, pleura, and mediastinum (n = 10, 10.1%). Some cases of misinterpretations occur as a failure to perform special stains, such as Periodic acid-Schiff stain not done in cases of the nasal polyp with fungal hyphae. Other errors occur by inadequate gross macroscopy examination when the pathologist did not select appropriate sections for microscopic examination. In these cases,

lymph node compromised by cancer, a polyp in the gallbladder, and breast carcinomas are not described in the first macroscopic description. These errors delay delivery results because they require a new specimen exam.

Delays in the report release may be considered as an error in the post-analytical [15] or analytical phase [16], and the turn-around time (TAT) should be used as an important quality measure in laboratories [32]. It is not uncommon for the pathologist to miss the perception that there is a patient waiting for his results; therefore, the cases should not remain for longer than necessary on the pathologist's desk [33]. Delays in TAT may be considered during the pre-analysis as delays in reception, gross examination, and material processing; during the analysis (in the diagnostic interpretation of the pathologist) or after the analysis, as the delay in typing and release of the reports to the patient. In a study performed with 713 cases of surgical pathology, 551 (77%) were released in 2 days and 162 (23%) in 3 days or more. From these, the majority of these cases were found to be pertaining to lungs, gastrointestinal tract, breasts, and samples of the genitourinary tract. Diagnosis of malignancy (including staging), consultations with other pathologists, freezing, and immunohistochemical analysis were associated with increased TAT in univariate analysis. In the multivariate analysis, the consultation with other pathologists, the diagnosis of malignancy, the use of immunohistochemistry, and the number of slides evaluated (11.3 when TAT > 2 days and 4.8 when TAT ≤ 2 days) remain as significantly associated with increased TAT. Despite CAP recommendation of an analytical response time of 2 days or less for most routine cases, the authors conclude that cancer care institutions should have a TAT longer than other services [34].

In post-analytical phase, errors include typographical errors, and in some cases, it can lead to catastrophic consequences, when the expression "cancer is present" instead of "cancer is not present." Another error in this phase included erroneous or missing non-diagnostic information, computer formatting, or transmission [29]. Besides that, some expressions can lead to confusing interpretations. It is broadly used in some expressions or phrases to communicate varying degrees of diagnostic certainty, for example, "cannot rule out," "consistent with," "highly suspicious," "favor," "indefinite for," "suggestive of," and "worrisome for." Lindley, Gillies, and Hassell evaluated 1500 surgical pathology reports and found 35% of these expressions, with wide variation in the percentage of certainty clinicians assigned to the phrases studied. The most commonly used phrases were "consistent with" (50%) and "suggestive of" (39%). The authors believe that the reasons for use for this expression may include contradictory or low probability staining results, inconsistency in clinical data, uncertain criteria in the medical literature, quantity of sample or abnormality, and possibly a concern with medicolegal consequences for an over- or under-diagnosis.

Nakhleh and Zarbo describe the amended reports from 359 laboratories, 96% of the USA, participants in the 1996 Q-Probes quality improvement program of the College of American Pathologists. A total of 3147 amended reports from 1,667,547 surgical pathology specimens accessioned in the study. They describe a median of amended reports was 1.5/1000 cases; of these, 19.2% were issued to correct patient identification errors, 38.7% to change the originally issued final diagnosis, 15.6% to change a preliminary written diagnosis, and 26.5% to change clinically significant information other than the diagnosis. The error detection was most commonly precipitated for a request from a clinician to review a case (20.5%) [35].

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4. Looking for solutions

Perkins [36] considers that the disclosure of errors in pathology is complicated by factors intrinsic to the specialty. The first barrier, as already mentioned, is the definition of error. Another concern is that the patient does not understand the nature of the error or even that the clinician is unable to explain it adequately to the patient. Even more complex is the situation that involves the discovery of the error of another individual: when the pathologist or the head of the laboratory discovers an error of a technician/ pathologist in their laboratory or external laboratories, or even when the pathologist discovers an error of a clinician from the same organization. Therefore, when disclosing an error, the pathologist must consider the potential impact on their professional relationships. It is difficult sometimes to define an error because there exists a great variability in definitions used in the literature. The most commonly utilized is a classification in pre-analytical, analytical, and post-analytical phases, but we note that the errors can overlap between these categories. For example, change of specimen can occur in pre-analytical and analytical phase. Incorrectly described laterality or anatomic sites may occur in any step at the laboratory. Because of that, the comparison of studies in literature can be difficult, as the authors used different definitions in their studies. We described a risk assessment of laboratory errors in surgical pathology in a fishbone diagram (**Figure 5**).

One factor conferred to the increase in the number of medical errors is the excessive decentralization of patient care. Since the patient may have several professionals working in different contexts and none with access to the complete information, the physician would work in a situation of greater susceptibility to error [1]. The lack of complete information is critical in pathology, where many cases depend on correct, clear, and complete clinical information for adequate clinical-pathological correlation. In some cases, radiological or laboratory correlation is required. In soft tissue and bone neoplasms, it is important that the pathologist

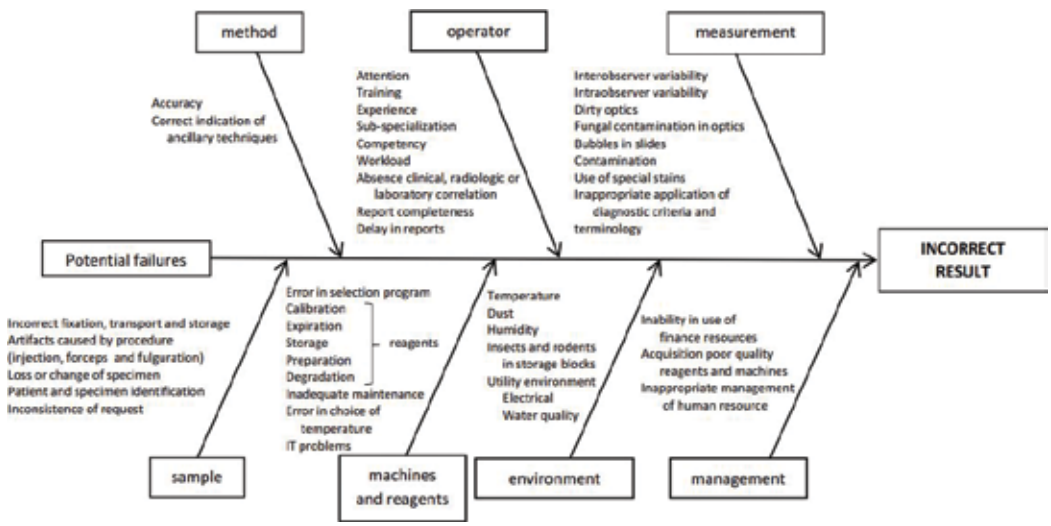

Figure 5. Risk assessment using a fishbone diagram.

is able to interpret radiological exams. The correlation with laboratory data is fundamental for interpretation of hepatic biopsies and to define etiology of hepatitis.

In 2016, CAP, the Laboratory Quality Center, and the Association of Directors of Anatomic and Surgical Pathology convened a panel of experts to develop a guideline to help define the role of case reviews in surgical pathology and cytology. The main recommendations cited in the document, with strong agreement among the participants were: (1) pathologists should develop procedures for the evaluation of selected cases in order to detect divergences and possible interpretation errors, (2) pathologists should conduct case reviews timely to prevent negative impacts on patient care, (3) pathologists should have review procedures of cases relevant to their practice, as well as continuously monitor and document the results of case reviews, and (4) if case reviews show unsatisfactory concordances for a defined case type, the pathologists should take action to improve diagnostic compliance. The situation may become a little more problematic in places where only one pathologist is responsible for all cases; almost all published data refer to situations in which there is a second pathologist responsible for the review. The authors understand that there may be a value addition when the pathologist himself revises his cases in the second moment; however, there are not enough data in the literature. Each laboratory should develop written procedures and record the results of its departmental review studies. According to the authors, the causes for low agreement within and among anatomopathological groups are multiple, but two factors need to be discussed. Some diagnoses have intrinsically greater variation between observers, and these differences should be considered. Furthermore, the histological diagnosis is dynamic and different terminologies can be used for the same disease. If a poor interobserver agreement is evidenced, methods for improvement should be implemented, such as consensus conferences, images for comparison, and so on; however, the quality of evidence is very low regarding the best method of improvement. The authors consider that best practices may differ according to the characteristics of the disease, individual practices, and complementary tests available [37].

Smith and Raab [9] describe how to use the Lean A3 quality control method in surgical pathology. Under the Lean method, a management philosophy developed by Toyota Motor Corp., pathologists develop activities, that is, examination of slides, diagnostics, and preparation of reports from paths through the sequential flow of the sample, with connections, represented by the individuals with whom the pathologist communicates. At all stages, there is the possibility of error, and quality improvements should focus on repairing these failures. The A3 method is based on defining a problem, analyzing its causes, aiming at an ideal practice, and providing an improvement plan [9]. Other authors have also used industrial techniques, such as the Six Sigma, with excellent results in error reduction [16, 38]. Examples of their measures were as follows: meetings with the clinical teams responsible for delivering the material to correct the inadequacy of the samples and intradepartmental meetings, in which employees actively participated in the discussions about the errors and their solutions. In the pre-analytical phase, the authors established a double-check system of the material, with the work divided into successive stages, and at each stage, all specimens were listed and checked by two team members, from receipt to material processing, and were subjected to the supervision of a quality control unit [16].

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In a review article by Ellis and Srigley [39], the authors emphasized the importance of structured and standardized reports for the improvement of diagnostic quality. Standardized reports can provide data that contribute to quality improvement programs in health care and, when combined with other health data sources, provide important information for monitoring, improvement, possible interventions, and benefit analyses in services offered to the population. The standardization of reports has proved to be particularly important in oncological diagnoses, which can generate much information with epidemiological impacts. The International Collaboration on Cancer Reporting maintains the guidelines and all the necessary parameters in the histopathological report at http://www.iccr-cancer.org/datasets to guide clinical management, as well as to provide prognostic information for several cancers; the guidelines panel is a result of a six-week public consultation conducted by a Dataset Authoring Committee, with multidisciplinary experts. Lehr and Bosman [33], in an article about the communication skills of pathologists, discourage the excess of additional notes on artifacts from improper pre-laboratory handling, such as incorrect fixation due to electrocautery, and so on. The authors advise that if the problems become recurrent, a letter to the material source services with guidelines may help to improve the specimens.

Nakhleh et al. [37] state that it is natural to wish to use data from case reviews to measure the quality of a pathology laboratory; however, now, it is not clear how best to interpret these results, which should not be used to compare the quality between two different laboratories. There are some limitations that may explain such facts: the sources of error, as well as their definitions, and the methods used for their measurement, which may differ between laboratories. Its clinical impacts may be different. The sensitivity of the evaluation method is not controlled and is unknown; in addition, the expected performance points are not well defined.

The errors in anatomical pathology have been screened in an internal assessment (review of diagnoses, correlation review of cytological and histological diagnoses, or between frozen section and permanent diagnoses, clinicopathological conference review of incoming cases, and intradepartmental cases or an intradepartmental consultation). The external assessment can be done across with regard to participation in quality assurance programs or medicolegal claims. Some authors recommended that two pathologists sign-out every cancer diagnosis [40]; however, this entails greater manpower, a luxury not enjoyed by a few laboratory [29].

Raab et al. [41] performed a nonconcurrent cohort study to compare the effectiveness and usefulness of error screening using a targeted 5% random review process (selected by a laboratory information system) and a focused review process. The last was performed in three subspecialties: gastrointestinal subspecialty, bone and soft tissue, and genitourinary pathology. In this study, pathologists reviewed 7444 cases using a targeted 5% random review process and 380 cases using a focused review process and describes 195 (2.6% of reviewed cases) and 50 (13.2%) errors detected by the procedures, respectively ($p < 0.001$). The focused review process detected approximately four times much more errors than the targeted 5% random review process, despite this last process involving the examination of almost 20 times the number of specimens. Major errors detected by the first process was 27 (0.36%) and 12 (3.2%)

detected by focused review processes with statistical difference ($p < 0.001$). The authors concluded that the focused review detects a higher proportion of errors and may be more effective in strategies for errors screening.

In some cases, the pathologists consult extradepartmental experts to achieve the better diagnostic accuracy, and it is known that the diagnostic criteria vary according to the pathologist's experience. For this reason, it is common to use the same expert for various pathologists. The principal limitation of this approach is the high selectivity of the cases because only extraordinary cases must be evaluated by other pathologists, and this does not exclude apparently routine cases that must be false-negative [7]. Besides that, the use of expert consultants does not exclude the legal responsibility for the first pathologist. In these situations, called "vicarious liability," the first pathologist assumes legal responsibility for having chosen a negligent consultant [30].

5. Conclusions

Errors in Pathology laboratory can result in serious adverse patient outcomes, with catastrophic results. False-negative outcomes in oncologic diagnosis result in a dangerous delay in adequate treatment. As opposed, to false-positive diagnosis, the patient can be submitted to several unnecessary procedures, such as extensive surgical resections, radiation therapy, or chemotherapy. It is difficult to imagine in which of the scenes the impact is greater: the delay of imperative treatment or an unwanted treatment for a healthy patient. In both situations, the consequences can be devastating—adverse effects or mutilations in treatment without clinical indications, with possibly fatal consequences, besides medical and legal consequences for the pathologist or laboratory involved in the biopsy process, with serious risks to the credibility and reputation of the pathologist and the laboratory.

The aim of any pathology laboratory must be establishing procedures that optimize quality control, such as additional case reviews and review of their laboratory techniques, to reduce interpretive errors or discrepancies in pathology reports. The quality formation, knowledge, and experience of the pathologist is crucial for diagnostic accuracy and the greater investment of laboratories, greater than higher technologies, must be continuing medical education for these professionals.

The taboo around the diagnostic error in pathology should be broken. It is not possible to discuss the quality controls of laboratories without admitting the possibility of error. Investing in continuing medical education, with emphasis on patient safety, as well as on the training of new pathologists, with a critical view aimed at reducing errors, is an obligatory path in improving the pathology practice.

Conflict of interest

The authors declare that they do not have any conflicts of interest.

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Chapter 8

A Practical Way to ISO/GUM Measurement Uncertainty for Analytical Assays Including In-House Validation Data

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Abstract

In this contribution, we outline the estimation of measurement uncertainty of analytical assays in a practical way, according to the so-called reconciliation paradigm, by considering the heritance of uncertainties according to the ISO Guide to the expression of uncertainty in measurement (ISO/GUM) approach and the accuracy (bias and precision) study coming from the in-house method validation. A cause and effect analysis is performed by using the Ishikawa diagram or fishbone plot, consisting of a hierarchical structure reaching a final outcome that is the analytical result. The procedure is illustrated with a case study. This procedure may be very suitable for processing data in accreditation of routine assays.

Keywords: ISO/GUM approach, method validation, uncertainty measurement

1. Introduction

The quality of analytical results is crucial because future decisions will be based on them. Uncertainty [1] is a good indicator of this quality. For example, two measurements made with the same ruler on different days by different people would be equivalent depending on their individual uncertainties.

Quality assurance measurements are a formal requirement in most of the analytical laboratories. As a consequence, to ensure that laboratories provide quality data, they are under continuous pressure to demonstrate their fitness for purpose, i.e., by giving confidence levels on the results. Measurement uncertainty will show the degree of agreement among results.

This concept of measurement uncertainty will be applicable to many cases, besides of quality control and quality assurance in production, such as complying with and enforcing laws and regulations, conducting a basic research, calibrating standards and instruments or developing, maintaining, and comparing international and national physical reference standards.

The ISO Guide to the expression of uncertainty in measurement, also known as the ISO/GUM or "bottom-up" [1], is one of the best approaches to estimate the uncertainty of analytical procedures. This procedure, originally conceived for use in physical measurements, has been suitably adapted to chemical ones in the EURACHEM/CITAC (Cooperation on International Traceability in Analytical Chemistry) guide [2] "Traceability in Chemical Measurement." However, this approach is tedious, time-consuming and unrealistic from the analytical viewpoint because their principles are significantly different from current procedures applied in analytical chemistry dealing with matrix effects, sampling operations and interferences [3, 4]. A strategy for reconciling the information requirements of ISO/GUM approach and the information coming from in-house method validation has been described by Ellison and Barwick [5]. The use of "cause" and "effect" analysis is the key for estimating the uncertainty of an analytical assay. In practice, this approach is performed by using a cause and effect diagram called Ishikawa or fishbone plot [6], consisting of a hierarchical structure that culminates in the "analytical result." In order to carry out the cause and effect analysis, the specification equation for the result is of utmost importance. The factors appearing in the equation (that contribute to the uncertainty of the result) are the main branches of the fishbone plot. For each branch, secondary factors can be considered, and so on, until their contribution to the result uncertainty is negligible. Two additional main branches (Recovery and Precision) come from the method validation. Nevertheless, these approaches exhibit some risks. The blind consideration of uncertainties coming from different sources of variation may lead to "double counting" in some instances. The analysts have to clearly identify the relationships among the sources of uncertainty in order to avoid duplications. Also, some sources of uncertainty that can be evaluated in a unique set of experiments must be suitably combined.

The combined uncertainty of the analytical measurand is the heritance of the uncertainties of all contributing variables (x_i) involved in the specification relationship where the value of measurand (Z) is defined as

$$Z = F(x_1, x_2, \ldots x_n) \tag{1}$$

Thus, the general expression for the combined uncertainty of measurand according to the law of propagation of uncertainty is given by

$$u^2(Z) = \sum_{i=1}^{n} \left(\frac{\partial F}{\partial x_i}\right)^2 u^2(x_i) + 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} \left(\frac{\partial F}{\partial x_i}\right)\left(\frac{\partial F}{\partial x_j}\right) \operatorname{cov}\left(x_i, x_j\right) \tag{2}$$

When the specification function consists of products or ratios only, and the factors are considered to be independent, then

$$\begin{aligned} \left(\tfrac{u(Z)}{Z}\right)^2 &= \sum_{i=1}^{n} \left(\frac{u(x_i)}{x_i}\right)^2 \\ u_{rel}^2(Z) &= \sum_{i=1}^{n} u_{rel}^2(x_i) \end{aligned} \tag{3}$$

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But this uncertainty does not consider the uncertainty contributions due to the intermediate precision of the assay and the trueness evaluated from recovery experiments. Nevertheless, it is possible to include these ones into the specification relationship either directly or by using unit-value factors $\left(f_i = 1 \pm u(f_i)\right)$ which do not contribute to the measurand value, but do contribute to its uncertainty [7, 8]. Accordingly, the modified specification relationship turns to:

$$Z = \frac{F(x_1, x_2, \ldots x_n)}{R} f_{prec} \tag{4}$$

The new involved parameters are the recovery, R, and the intermediate precision of the assay, f_{prec}. These contributions are issued from the data of method validation study. Accordingly, the uncertainty of measurand can be written as:

$$u_{rel}^2(Z) = \sum_{i=1}^{n} u_{rel}^2(x_i) + u_{rel}^2(R) + RSD_{prec}^2 \tag{5}$$

At this step, the considerations regarding to the sources of uncertainties have to be taken into account in order to avoid either under- or over-estimations of the result uncertainty.

The specification relationship involves a given set of parameters depending on the analytical procedure applied. Common factors are: mass determinations (obviously for sample weight and used standards), volumetric measurements (glassware and other devices delivering volume), analyte concentration coming from indirect calibration, and the precision and recovery of the analytical assay established in the validation study.

In the following, these factors will be outlined and their uncertainties will be discussed.

1.1. Uncertainty of sample mass

In a typical mass determination, the analytical balance is zeroed with the empty container on the pan, and the container is the filled and weighed. In this case, the uncertainty of mass measurements (without considering buoyancy) is given by [9]

$$u(m) = \sqrt{S_r^2 + S_{env}^2 + \frac{2}{3}a_L^2 + \frac{m^2 a_T^2 (\Delta T)^2}{9} + u_{CAL}^2} \tag{6}$$

where $S_r^2 + S_{env}^2$ is the variance of replication (repeatability and environmental variances) expressed as an weighting intermediate precision, a_L is the linearity specification of the balance, a_T is the sensitivity temperature coefficient, ΔT is the difference between the room temperature and the calibration temperature (20°C) and u_{cal} is the standard uncertainty for balance calibration.

Because the intermediate precision study is carried out for the entire analytical assay at the validation stage, individual contributions to the intermediate precision (here, weighting intermediate precision) cannot be taken into account for avoiding redundant counting of uncertainty. Thus, the uncertainty of mass will include the uncertainty contribution of lack

of linearity of balance, the uncertainty due to temperature effect and the calibration uncertainty

$$u(m) = \sqrt{\frac{2}{3}a_L^2 + \frac{m^2 a_T^2 (\Delta T)^2}{9} + u_{CAL}^2} \tag{7}$$

1.2. Uncertainty of glassware volume

As R. Kadis pointed out [10], the evaluation of uncertainty of volumetric measurements consists of three kinds of contributions: specification limits for the glassware of a given class, repeatability of filling the glassware to the mark and temperature effects. Again, in order to avoid double counting and uncertainty redundancy, the precision of filling the flask is not considered here; thus, the uncertainty in the volume measurement is given by

$$u(V) = \sqrt{\frac{a_{TOL}^2}{6} + \frac{\chi^2 V^2 (\Delta T)^2}{3}} \tag{8}$$

where a_{TOL} is the tolerance for a given class, χ is the dilatation coefficient for the filling liquid ($2.1 \times 10^{-4}\ \mathrm{K}^{-1}$ for water), and ΔT as indicated earlier.

1.3. Uncertainty of concentration coming from calibration

Generally, in routine analysis, analytical determinations involve instrumental method where indirect calibration is applied. Common scenarios include external calibration, standard addition calibration (in case of matrix effects) and internal standard calibration (when intrinsic analytical signal variations appear or analyte losses may occur owing to sample preparation procedures [11]).

In case of linear calibration, the calibration straight line is established by preparing calibration standards. The primary stock standard solution is made by weighing the suitable mass of standard (m_{std}), of a given purity (P) in the corresponding volume of solvent (V_s)

$$C_{std} = \frac{m_{std} P}{V_s} \tag{9}$$

But this concentration has an uncertainty derived from the uncertainty in the weighting, in its purity and in the uncertainty of the glassware. The working standard solutions are prepared by diluting a volume (V_i) of the stock standard solution to a final volume V_f. So, the concentration of any calibration standard is given by

$$C_i = C_{std} \frac{V_i}{V_f} = \frac{m_{std} P V_i}{V_s V_f} \tag{10}$$

and has an uncertainty that can be suitably calculated. However, when applying ordinary least-squares techniques (simple linear regression), three requisites have to be fulfilled [12]:

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- The independent variable x, is free from error $(\varepsilon(x) = 0)$ or at least, $\varepsilon(x) << \varepsilon(Y)$.
- The error associated to Y variable, is normally distributed, $N(0, \sigma^2)$.
- The variance of the Y variable, σ^2, remains uniform in the whole range of x (homoscedasticity).

In our case, the independent variable is the concentration of standard (C_i) and the Y variable is the analytical signal. In a typical case of multipoint calibration (external or internal), the three requirements mentioned above applies, and the ordinary least-squares procedure gives the calibration straight line $\widehat{Y}_i = b_0 + b_1 C_i$. The unknown analyte content is predicted from interpolation of the sample response signal Y_0 according to

$$C_{cal} = \frac{Y_0 - b_0}{b_1} \tag{11}$$

whose uncertainty can be estimated from the variance propagation law:

$$\begin{aligned} u^2(C_{cal}) &= \left(\frac{\partial C_{cal}}{\partial Y}\right)^2 u^2(Y_0) + \left(\frac{\partial C_{cal}}{\partial b_0}\right)^2 u^2(b_0) + \left(\frac{\partial C_{cal}}{\partial b_1}\right)^2 u^2(b_1) + 2\left(\frac{\partial C_{cal}}{\partial b_0}\right)\left(\frac{\partial C_{cal}}{\partial b_1}\right) \operatorname{cov}(b_0, b_1) \\ &= \frac{u^2(Y_0)}{b_1^2} + \frac{u^2(b_0)}{b_1^2} + \frac{(Y_0 - b_0)^2}{b_1^4} u^2(b_1) + \frac{(Y_0 - b_0)}{b_1^3} \operatorname{cov}(b_0, b_1) \\ &= \frac{u^2(Y_0)}{b_1^2} + \frac{u^2(b_0)}{b_1^2} + \frac{(Y_0 - b_0)^2}{b_1^4} u^2(b_1) - \frac{(Y_0 - b_0)}{b_1^3} \overline{C} u^2(b_1) \end{aligned} \tag{12}$$

where $\overline{C} = \frac{1}{N}\sum_{i}^{N} C_i$, N being the number of calibration points.

Eq. (10) can be rearranged to give the well-known formula recommended by EURACHEM [2]:

$$u(C_{cal}) = \frac{s_{y/x}}{b_1} \sqrt{\frac{1}{m} + \frac{1}{N} + \frac{\left(C_{cal} - \overline{C}\right)^2}{\sum_{i=1}^{N} \left(C_i - \overline{C}\right)^2}} \tag{13}$$

Here, $s_{y/x}$ is the residual standard deviation of the regression line, m is the number of replications measuring the sample signal and N the number of calibration points [13].

Aside from the calibration uncertainty, an additional uncertainty contribution can be considered from the preparation of standards as indicated in Eq. (8) and may be accounted separately in the uncertainty budget:

$$\frac{u^2(C_i)}{C_i^2} = \frac{u^2(m_{std})}{m_{std}^2} + \frac{u^2(P)}{P^2} + \frac{u^2(V_i)}{V_i^2} + \frac{u^2(V_s)}{V_s^2} + \frac{u^2(V_f)}{V_f^2} \tag{14}$$

Thus, the uncertainty of concentration is given by the uncertainty on sample analyte concentration coming from calibration, and the uncertainty due to the preparation of standards.

1.4. Uncertainty of the analytical assay from the in-house data of method validation (precision and trueness)

Intralaboratory assessment of method accuracy encompasses both precision and trueness study.

As EURACHEM guide advices [2], "the precision should be estimated as far as possible over an extended period of time." This may be accomplished by performing a between-day laboratory precision study. This precision study is carried out either by analyzing a typical sample, a quality control check sample or a validation standard [14] in "intermediate precision" conditions. Intermediate precision is the intralaboratory global precision under varied conditions as expected within a laboratory in a future assay. Accordingly, if a between-day precision study is carried out by spacing out the measurement days in such a way that the analysts, the apparatuses, glassware, stock solutions…really change, the precision estimation (from ANOVA) is a suitable "intermediate precision" estimation [14], leading to an evaluation of intermediate precision uncertainty, u_{IP}.

Again, according to EURACHEM [2], the trueness (bias) study can be performed

- by repeated analysis of a certified reference materials (CRM), using the complete measurement procedure;
- by comparing the results of analyzed samples against a reference method; and
- by applying recovery assays, using spiked placebos (validation standards) when available or spiked samples instead, and evaluating the recovery.

Thus, an estimation of the uncertainty of bias or recovery is calculated.

Both precision and trueness studies have to be carried out at least at three analyte concentration levels (low, medium and high) in order to cover the full range of analyte concentration indicated in the method scope.

In his excellent paper, Kadis [13] discussed the double counting risk in the uncertainty budget when calibration uncertainty is considered together with the precision uncertainty. The term $\frac{s_{x/y}}{b_1 m}$ in Eq. 13 features the estimated precision of the analyte concentration in the calibration experiment. The estimated precision (from in-house validation) considers all the sources of variability, including calibration, therefore the contribution of $\frac{s_{x/y}}{b_1 m}$ in the calibration uncertainty is redundant. Accordingly, the first term under the radical in Eq. (13) must be omitted to avoid double counting, or alternatively, the precision uncertainty can be omitted in the budget. Moreover, the recovery uncertainty includes the precision of the analyte mean value, which is used in the computation of recovery. Thus, some authors do not include the precision uncertainty together with the recovery uncertainty in the budget [13].

The use of cause and effect diagrams for designing the uncertainty budget including the in-house validation data is illustrated in the following worked example selected as case study.

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2. Fluorimetric determination of quinine in tonic water

This working example has been prepared from the papers of O'Reilly [15] and González and Herrador [14], and deals with the determination of quinine in tonic water samples from fluorescence measurements. Solutions that contain quinine in acid medium (0.05 M sulfuric acid) show fluorescence with a maximum excitation wavelength at 350 nm and a maximum emission wavelength at 450 nm. The determination of quinine in tonic water samples is carried out according to the following procedure [16]: 1 mL of tonic water (previously degassed by 15 min sonication in an ultrasonic bath) was pipetted into a 100 mL volumetric flask and dilute to the mark with 0.05 M sulfuric acid. The fluorescence intensity of this solution is measured in a fluorescence spectrometer in 10 mm pathway quartz cells at 350 nm excitation wavelength and at 450 nm emission wavelength. The quinine concentration is interpolated in the corresponding calibration curve. All analytical operations were done at 20 ± 4°C.

The specification equation for estimating the quinine concentration (mg/L) in tonic water samples is given by

$$Z = \frac{C_{cal} V}{V_0 R} f_{prec} \tag{15}$$

where C_{cal} is the value (mg/L of quinine) interpolated in the calibration curve from the measured fluorescence intensity of the assay, V is the volume of the assay (100 mL), V_0 is the sample volume (1 mL), R is the recovery of the assay and f_{prec} is the factor corresponding to the assay precision which has a value of 1, but an uncertainty equals to the precision standard deviation of the Z measurement. Recovery and precision data are taken from the in-house validation study of the method. The corresponding cause and effect Ishikawa diagram is depicted in **Figure 1**.

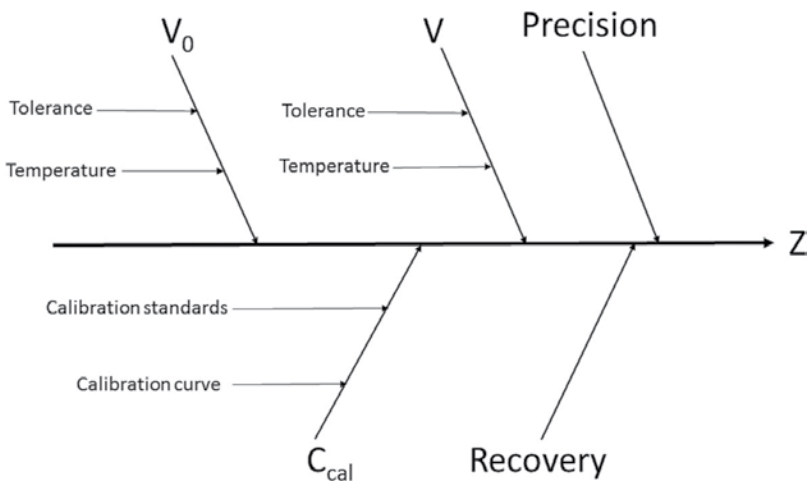

Figure 1. Cause and effect diagram for the fluorimetric determination of quinine in tonic water.

According to the fishbone plot, the uncertainty budget is as follows:

$$u_{rel}^2(Z) = u_{rel}^2(C_{cal}) + \sum_{i=1}^{5} u_{rel}^2(C_i) + u_{rel}^2(V) + u_{rel}^2(V_0) + u_{rel}^2(R) + RSD_{prec}^2 \quad (16)$$

Now, each uncertainty contribution is studied and evaluated.

2.1. Uncertainty coming from calibration and standards

In order to establish the corresponding calibration curve, a stock solution of quinine was prepared by weighing 121.6 mg of quinine sulfate dihydrate with a minimum purity of 99% (or 99.5 ± 0.5%) and dissolving and diluting 0.05 M sulfuric acid to 1000 ml in a volumetric flask. The concentration of this stock solution corresponds to 100 mg/L of quinine base.

Six working standards solution covering from 0.2 to 1.2 mg/L quinine were prepared by pipetting 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL of the stock solution and diluting with 0.05 M sulfuric acid in a 50 mL volumetric flask, leading to concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/L quinine, respectively. The fluorescence intensity of each working standard at 350 nm excitation wavelength and at 450 nm emission wavelength was measured in triplicate. The results are shown in **Table 1**.

Fluorescence intensities show a linear behavior against the quinine concentration according to a calibration straight line with a correlation coefficient of about 0.999, and the following features:

$$b_1 = 784.76,\ \ b_0 = 13.67,\ \ s_{x/y} = 3.15,\ \ N = 18,\ \ \overline{C} = 0.7,\ \ \sum_{i=1}^{18} \left(C_i - \overline{C}\right)^2 = 2.1$$

The corresponding calibration uncertainty assuming that the analytical signal is measured in triplicate (m = 3) from Eq. (11) is given by:

Working standard solution, mg/L	**Fluorescence, AU**		
	Trial 1	**Trial 2**	**Trial 3**
0.2	171	172	171
0.4	327	328	330
0.6	484	481	481
0.8	642	640	643
1.0	800	798	799
1.2	954	958	955

Table 1. Fluorescence intensities (UA) for the five working standard solutions, measured in triplicate.

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$$u(C_{cal}) = 4 \times 10^{-3}\sqrt{\frac{1}{3} + \frac{1}{18} + \frac{(C_{cal} - 0.7)^2}{2.1}} \tag{17}$$

Uncertainty due to preparation of working calibration standards is computed from Eq. (12). The uncertainty of the standard mass can be evaluated according to Eq. (7). In our case, the balance specifications were: Linearity (a_L): 0.2 mg. Sensitivity temperature coefficient (a_T): $2.5 \times 10^{-6}\,K^{-1}$. The calibration certificate indicates an expanded uncertainty of 8×10^{-4} g with a coverage factor, k = 2. Because the analytical operations are performed at 20° ± 4°C and ΔT = 4°. Thus, we have:

$$\begin{aligned} u(m_{std}) &= \sqrt{\frac{2}{3}(0.2)^2 + \frac{m_{std}^2\left(2.5 \times 10^{-6}\right)^2(4)^2}{9} + (0.4)^2} \\ &= \sqrt{0.187 + 1.11 \times 10^{-11} m_{std}^2}; 0.432mg \end{aligned}$$

The uncertainty of purity is evaluated from the specification: 0.995 ± 0.005 and assuming a rectangular distribution. Thus, $u(P) = \frac{0.005}{\sqrt{3}} = 2.9 \times 10^{-3}$. Uncertainty in volumes (from pipettes or volumetric flasks) are calculated from Eq. (8). The corresponding tolerances for glassware laboratory (Class A) are gathered in **Table 2**, except for the class A graduated pipette of 1 mL (for delivering volumes from 0.1 to 0.6 mL) which is ±0.006.

In the case of working standards, $V_i = 0.1, 0.2, 0.3, 0.4, 0.5$ and 0.6 mL for each working solution, $V_s = 1000\,\text{mL}$ and $V_f = 50\,\text{mL}$. Accordingly, we get

$$u(V_1) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.1^2 (4)^2}{3}} = 2.45 \times 10^{-3}$$

$$u(V_2) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.2^2 (4)^2}{3}} = 2.45 \times 10^{-3}$$

$$u(V_3) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.3^2 (4)^2}{3}} = 2.46 \times 10^{-3}$$

$$u(V_4) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.4^2 (4)^2}{3}} = 2.46 \times 10^{-3}$$

$$u(V_5) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.5^2 (4)^2}{3}} = 2.46 \times 10^{-3}$$

$$u(V_6) = \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 0.6^2 (4)^2}{3}} = 2.47 \times 10^{-3}$$

$$u(V_s) = \sqrt{\frac{(0.3)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 1000^2 (4)^2}{3}} = 0.5$$

$$u(V_f) = \sqrt{\frac{(0.05)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 50^2 (4)^2}{3}} = 0.0317$$

Level	Theoretical concentration	Predicted concentration				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	66	65.33	66.81	67.44	65.72	66.61
1	66	65.38	66.79	67.48	65.70	66.36
1	66	65.22	66.72	67.48	65.88	66.70
2	83	84.49	82.83	82.65	82.30	83.74
2	83	84.53	82.77	82.70	82.51	83.82
2	83	84.60	82.92	82.56	82.48	83.65
3	100	100.25	101.36	99.98	98.84	99.60
3	100	100.20	101.44	100.02	98.93	99.77
3	100	100.32	101.50	99.87	98.75	99.82

Table 2. Predicted concentration of the spiked placebos expressed in mg/L quinine.

The total relative uncertainty of the working standards can be evaluated by avoiding multiple counting as follows:

$$\begin{aligned} u_{rel}^2(C_i) &= \frac{u^2(m_{std})}{m_{std}^2} + \frac{u^2(P)}{P^2} + \sum_{i=1}^{6} \frac{u^2(V_i)}{V_i^2} + \frac{u^2(V_s)}{V_s^2} + \frac{u^2(V_f)}{V_f^2} \\ &= \frac{0.432^2}{121.6^2} + \frac{\left(2.9 \times 10^{-3}\right)^2}{0.995^2} + \frac{\left(2.45 \times 10^{-3}\right)^2}{0.1^2} + \frac{\left(2.45 \times 10^{-3}\right)^2}{0.2^2} \\ &\quad + \frac{\left(2.46 \times 10^{-3}\right)^2}{0.3^2} + \frac{\left(2.46 \times 10^{-3}\right)^2}{0.4^2} + \frac{\left(2.47 \times 10^{-3}\right)^2}{0.5^2} \frac{\left(2.47 \times 10^{-3}\right)^2}{0.6^2} \\ &\quad + \frac{0.5^2}{1000^2} + \frac{0.0317^2}{50^2} \\ &= 9.18 \times 10^{-4} \end{aligned} \tag{18}$$

2.2. Uncertainty of assay and sample volumes

The uncertainties of the assay and sample volume are also estimated from Eq. (8) and tolerances of **Table 2**:

$$\begin{aligned} u(V) &= \sqrt{\frac{(0.08)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 (100)^2 (4)^2}{3}} = 0.058 \\ u_{rel}^2(V) &= \frac{0.058^2}{100^2} = 3.36 \times 10^{-7} \\ u(V_0) &= \sqrt{\frac{(0.006)^2}{6} + \frac{\left(2.1 \times 10^{-4}\right)^2 (1)^2 (4)^2}{3}} = 2.5 \times 10^{-3} \\ u_{rel}^2(V_0) &= \frac{\left(2.5 \times 10^{-3}\right)^2}{1^2} = 6.25 \times 10^{-6} \end{aligned} \tag{19}$$

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2.3. Uncertainty of precision and trueness from in-house validation

The study of precision (intermediate precision) and trueness (recovery of assay) for the fluorimetric determination of quinine in tonic water was performed by using validation standards (spiked placebos) as indicated by González and Herrador [16]. Validation standards of quinine in tonic water matrix were prepared at low (66 mg/L), medium (83 mg/L) and high level (100 mg/L), covering the whole range of analyte concentrations (from 80 to 120% of 83 mg/L of quinine that is the recommended value of quinine in tonic waters by the FAD [17]). Both precision and trueness study was performed by predicting the actual concentrations of the three spiked placebos according to the recommended fluorimetric procedure for quinine determination. Measurements were made on 5 days for each validation standard with three replications of the assay. The results obtained are presented in **Table 3**.

The best way to estimate both the uncertainty contribution of intermediate precision and the recovery (or bias) of the analytical assay when validation standards are available, is using ANOVA at a given concentration of the validation standard, namely *T*, considering p different conditions (5 days in this case) and n replications (3 days in this case). From the ANOVA results (within conditions variance, S_W^2, between conditions variance, S_B^2, and total mean, $\overline{\overline{x}}$), the values of variance due to the condition (here, days), $S_{condition}^2$, the variance of repeatability, S_r^2, the variance of intermediate precision, S_{IP}^2 as well as the bias and recovery together with their uncertainties can be easily computed [14, 18]:

$$S_{condition}^2 = \frac{S_B^2 - S_W^2}{n};\ \ S_r^2 = S_W^2;\ \ S_{IP}^2 = S_r^2 + S_{condition}^2; RSD_{IP}^2 = \frac{S_{IP}^2}{\overline{\overline{x}}^2}$$
$$R = \frac{\overline{\overline{x}}}{T};\ \ u^2(R) = \frac{S_{IP}^2 - \frac{n-1}{n}S_r^2}{pT^2};\ \ u_{rel}^2(R) = \frac{S_{IP}^2 - \frac{n-1}{n}S_r^2}{p\overline{\overline{x}}^2} \tag{20}$$

Class A glassware.	Capacity, mL	Tolerance, mL
Burette	50	± 0.05
	25	± 0.03
	10	± 0.02
Pipette	40–50	±0.05
	15–30	±0.03
	8–10	±0.02
	3–7	± 0.01
	1–2	±0.006
Volumetric flask	1000	±0.3
	500	±0.15
	100	±0.08
	50	±0.05
	25	±0.03

Table 3. Tolerances for class A laboratory glassware.

Nominal concentration (T, mg/L quinine)	$\bar{\bar{x}}$	RSD^2_{IP}	R	$u^2_{rel}(R)$
66	66.38	1.65×10^{-4}	1.0057	3.28×10^{-5}
83	83.24	1.13×10^{-4}	1.0029	2.25×10^{-5}
100	100.04	8.87×10^{-5}	1.0004	1.77×10^{-5}

Table 4. Relative precision and uncertainty of recovery for the three validation standards in the fluorimetric determination of quinine in tonic water.

Thus, values of RSD^2_{IP} and $u^2_{rel}(R)$ are obtained for each spiked placebo. These data are presented in **Table 4**. A significance test has been used to evaluate if the recovery is significantly different from unity for each spiked placebo:

$$t = \frac{|1 - R|}{u(R)}$$

This value is then compared with the two-tailed critical value of tabulated Student-t statistic for np-1 degrees of freedom (14 in our case) at a 95% confidence level ($t_{crit}(14, 95\%) = 2.145$). For the three studied validation standards, recoveries were significantly equal to unity, and we can set $R = 1$ in all cases.

As can be seen in Eq. (20), the value of RSD^2_{IP} contains $u^2_{rel}(R)$ and accordingly, as it was indicated above, we can neglect the contribution $u^2_{rel}(R)$ in the uncertainty budget. The value of relative precision for the determined quinine concentration is taken as $RSD^2_{prec} = \frac{RSD^2_{IP}}{m}$ (here, m = 3).

Now, all contributions of specification factors have been included in the budget. Consider now that a sample of tonic water (Schweppes) has been analyzed by following the recommended procedure. The response is measured in triplicate (m = 3), leading to a fluorescence intensity (AU) of 617.5, 618.1 and 616.7. The mean value is $Y_0 = 617.43$ that corresponds to a quinine concentration of the assay of $C_{cal} = \frac{617.43 - 13.67}{784.76} = 0.76936$. Accordingly, the value of calibration uncertainty from Eq. (17), but neglecting the radical term 1/3 in order to avoid double counting, gives $u(C_{cal}) = 10^{-3}$ and $u^2_{rel}(C_{cal}) = 1.7 \times 10^{-6}$. The concentration of quinine in the sample according Eq. (15) with $R = 1$ and $f_{prec} = 1$ is Z = 76.936 ppm. We can interpolate this value in **Table 4** in order to estimate the corresponding $RSD^2_{IP} = 1.31 \times 10^{-4}$ that leads to $RSD^2_{prec} = \frac{1.31 \times 10^{-4}}{3} = 4.38 \times 10^{-5}$. Then, by applying Eq. (16), disregarding the recovery contribution, we get

$$\begin{aligned} u^2_{rel}(Z) &= u^2_{rel}(C_{cal}) + u^2_{rel}(C_i) + u^2_{rel}(V) + u^2_{rel}(V_0) + RSD^2_{prec} \\ &= 1.7 \times 10^{-6} + 9.18 \times 10^{-4} + 3.37 \times 10^{-7} + 6.25 \times 10^{-6} + 4.38 \times 10^{-5} \\ &= 0.00097 \end{aligned}$$

Thus, $u_{rel}(Z) = 0.03115$ and $u(Z) = 76.936 \times 0.03115 = 2.396$. By assuming a Gaussian coverage factor of 95% confidence k = 2, the expanded uncertainty is $U(Z) = 4.792$ and the quinine concentration of Schweppes tonic water sample is $Z = 77 \pm 5 ppm$.

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3. Selected applications in tabular form

A more detailed picture of most recent selected papers about the "Guide to the Expression of Uncertainty in Measurement" is depicted in **Table 5**, giving an idea of the importance and

Content	Authors	Ref.
General overview about concepts, models, methods, and computations that are commonly used for the evaluation of measurement uncertainty, and their application in realistic examples drawn from multiple areas of science and technology.	Possolo and Iyer, 2017	[19]
A complete procedure to encompass an uncorrected bias into the expanded uncertainty with a fixed coverage probability.	Synek, 2017	[20]
Reported scientific uncertainties by analyzing 41,000 measurements of 3200 quantities from medicine, nuclear and particle physics, and interlaboratory comparisons ranging from chemistry to toxicology.	Bailey, 2016	[21]
The GUM revision: the Bayesian view toward the expression of measurement uncertainty.	Lira, 2016	[22]
Comparing methods for evaluating measurement uncertainty given in the Joint Committee for Guides in Metrology 'Evaluation of Measurement Data' documents.	Stant et al., 2016	[23]
In pursuit of a fit-for-purpose uncertainty guide: the move away from a frequentist treatment of measurement error to a Bayesian treatment of states of knowledge is misguided.	White, 2016	[24]
Three controversies faced in the development of GUM document: (i) the acceptance of the existence of 'true values', (ii) the association of variances with systematic influences and (iii) the representation of fixed but unknown quantities by probability distributions.	Willink, 2016	[25]
A new way to express uncertainty of measurement is proposed that allows for the fact that the distribution of values attributed to the measurand is sometimes approximately lognormal and therefore asymmetric around the measurement value.	Ramsey and Ellison, 2015	[26]
Revision of the GUM: reasons why the Guide needed a revision, and why that revision could not go in a direction different from the one that it has been taken.	Bich, 2014	[27]
Validating the applicability of the GUM procedure.	Cox and Harris, 2014	[28]
Evolution in thinking and its impact on the terminology that accompanied the development of the GUM	Ehrlich, 2014	[29]
The developments in uncertainty concepts and practices that led to the third edition of the Eurachem Guide on uncertainty evaluation.	Ellison, 2014	[30]
A review of monte carlo simulation using microsoft excel for the calculation of uncertainties through functional relationships, including uncertainties in empirically derived constants.	Farrance and Frenkel, 2014	[31]
Evaluation of mass measurements in accordance with the GUM. The importance of reporting calibration results in a compact way that is easily propagated down the traceability chain is also discussed.	Nielsen, 2014	[32]
Overview about statistical models and computation to evaluate measurement uncertainty.	Possolo, 2014	[33]
Discussion about recent situation in measurement science, and how to obtain a reliable measurement result using the expression of metrological traceability together with measurement uncertainty.	Imai,2013	[34]
A new strategy for the analytical validation based on the uncertainty profile as a graphical decision-making tool, and to exemplify a novel method to estimate the measurement uncertainty.	Saffaj et al., 2013	[35]

Content	Authors	Ref.
Monte Carlo approach for estimating measurement uncertainty using standard spreadsheet software.	Chew et al., 2012	[36]
General overview of the GUM and to show how the calculation of uncertainty in the measurand may be achieved through a functional relationship.	Farrance and Frenkel, 2012	[37]
Estimation of the measurement uncertainty in quantitative determination of ketamine and norketamine in urine using a one-point calibration method.	Ma et al., 2012	[38]
EURACHEM/CITAC workshop on recent developments in measurement uncertainty. Contains a selection of the contributed papers at this workshop and show how the evaluation of uncertainty is now being applied to a wide range of analyses.	Williams, 2012	[39]
Highlight some of the differences between the two concepts of total error and uncertainty but also to stress their main similarities.	Rozet et al., 2011	[40]
The assurance as a result of blood chemical analysis by ISO-GUM and Quality Engineering.	Iwaki, 2010	[41]
Managing quality vs. measuring uncertainty in the medical laboratory. The paper argues that total error provides a practical top-down estimate of measurement uncertainty in the laboratory, and that the ISO/GUM model should be primarily directed to and applied by manufacturers.	Westward, 2010	[42]
Comparison of the approach to measure uncertainties proposed in ISO 5725 and GUM from a statistician point of view.	Deldossi and Zappa, 2009	[43]
Utilizing the correlations between the N individual results, an equation is derived to combine the N individual uncertainties of N measurements. Using the newly derived equation including the correlation coefficient, three measurement uncertainties of three measurement results are combined as an example.	Nam et al., 2009	[44]
From GUM to alternative methods for measurement uncertainty evaluation.	Priel, 2009	[45]
Critical debate about the revision of the Guide to the expression of uncertainty in measurement.	Bich, 2008	[46]
Course aimed at developing understanding of measurement and uncertainty in the introductory physics laboratory. The course materials, in the form of a student workbook, are based on the probabilistic framework for measurement as recommended by the International Organization for Standardization in their publication GUM.	Buffler et al., 2008	[47]
Scientific discussion about measurement uncertainty and chemical analysis.	Kadis, 2008	[48]
Treatment of uncorrected measurement bias in uncertainty estimation for chemical measurements.	Magnusson and Ellison, 2008	[49]
A critical overview of the current doubtful practice on presentation of correlated data in the physics literature and in the scientific and technological databases.	Ezhela, 2007	[50]
A detailed step-by-step guide to analytical method validation, considering the most relevant procedures for checking the quality parameters of analytical methods.	González and Herrador, 2007	[9]
Development of the concept of uncertainty in measurement and the methods for its quantification from the classical error analysis to the modern approaches based on the GUM.	Kacher et al., 2007	[51]
Measurement uncertainty: top-down and bottom-up approach, tools for its determination uncertainty sources and practical examples.	Meyer, 2007	[52]
Critical review about calibration-, uncertainty-, and recovery-related documents from 10 consensus-based organizations.	Vanatta and Coleman, 2007	[53]
Evolution of the GUM: documents relating to the GUM planned by Joint Committee for Guides in Metrology.	Bich et al., 2006	[54]

Content	Authors	Ref.
Calculating uncertainty of measurement for serology assays by use of precision and bias.	Dimech et al., 2006	[55]
Comparison of ISO-GUM, draft GUM Supplement 1 and Bayesian statistics using simple linear calibration.	Kacher et al., 2006	[56]
Estimation of the measurement uncertainty of analytical assays based on the LGC/VAM protocol from validation data in the light of the study of precision, trueness and robustness.	González et al., 2005	[57]
Philosophy behind the GUM, and demonstrates, with a medical physics measurement example of how the GUM recommends uncertainties be calculated and reported.	Gregory et al., 2005	[58]
The limitations of the GUM for evaluating the uncertainty of indirect measurements. The propagation of distributions as the best way to evaluate the measurement. Uncertainty and the use of Monte-Carlo method for performing the propagation of distributions is outlined and discussed.	Herrador et al., 2005	[59]
Comparison of six commercial programs devoted to the estimation of measurement uncertainty for feasibility in order to be applied in routine chemical analysis.	Jurado and Alcázar, 2005	[60]
Treatment of bias in estimating measurement uncertainty.	O'Donnell and Hibbert, 2005	[61]
Statistical analysis of Consultative Committees of the International Committee of Weights and Measures (CIPM) key comparisons based on the ISO Guide.	Kacker et al., 2004	[62]
General overview of the uncertainty of measurement concept, with minimal metrological terminology, and also practical guidelines to assist pathology laboratories comply with this accreditation requirement.	White and Farrance, 2004	[63]
Approach to determine the overall uncertainty by combining the uncertainties of the individual results when the difference is statistically significant by GUM.	Choi et al., 2003	[64]
An appraisal on the guide to expression of uncertainty in measurement approach for estimating uncertainty.	Kristiansen, 2003	[65]
Critique of the Guide to the expression of uncertainty in measurement method of estimating and reporting uncertainty in diagnostic assays.	Krouwer, 2003	[66]
Effect of non-significant proportional bias in the final measurement uncertainty.	Maroto et al., 2003	[67]
Background of the GUM. The knowledge of the respective measurement and other fundamental aspects which have been included in the EA-4/02 requirements document published by the European co-operation for accreditation.	Kessel, 2002	[68]
Operational definitions of uncertainty taking into account the differences in the ways in which truth, uncertainty and error are conceived.	Hund et al., 2001	[69]
Approaches to the evaluation of uncertainties associated with recovery	Barwick and Ellison, 1999	[70]
Review of the concepts and practices of data quality in analytical chemistry in relation to uncertainty. It is addressed primarily to the bodies that will be responsible for the introduction of uncertainty into routine practice.	AMC, 1995	[71]
Future trends in analytical quality assurance, the evaluation of the quality of analytical results by estimation of their uncertainties. The present state-of-the-art is described, and the impact caused by the declaration of uncertainties in chemical results is foreseen.	Cortez, 1995	[72]
Critical reflexion about the uncertainty concept and its method for estimation.	Thompson, 1995	[73]
Guidelines for evaluating and expressing the uncertainty of NIST measurement results.	Taylor and Kuyatt, 1994	[74]

Table 5. Selected papers about the "Guide to the Expression of Uncertainty in Measurement (GUM)".

relevance of the topic in different fields. Emphasis is stressed on reviews and taking into account the high number of references available, the authors apologize for those they may have overlooked or inadvertently omitted. Selected applications about the estimation of uncertainty in volumetric glassware, analytical balance and calibration curves, as well as the evaluation of the measurement uncertainty in classical and instrumental techniques are shown in **Tables 6** and **7**. **Figure 2** shows the number of publications cited per year, whereas in **Figure 3**, the number of paper cited by journal for the most cited journals appears.

Content	Reference	Ref.
Volumetric glassware		
Uncertainty on using graduated volumetric glassware for the concentration of samples (concentration tube) and its effect on measurement accuracy.	Matsuda et al., 2015	[75]
Experimental study on evaluation of uncertainty in volumetric measurement: pipettes, graduated pipettes, graduated burettes, volumetric flasks and micropipettes used in various analytical and biological studies.	Mukund et al., 2015	[76]
Influencing factors in uncertainty measurement that affect mass and volume determination. Technical specification of an analytical balance such as: readability, repeatability, linearity, off-center loading and hysteresis and for volumetric glassware: repeatability, readability, temperature coefficient of sensitivity, temperature scattering, meniscus reading and environmental conditions (temperature and humidity) are considered.	Rahman et al., 2015	[77]
Analysis of the results obtained in the calibration of electronic analytical balances.	Valcu and Baicu, 2012	[78]
Influence quantities for the uncertainty of a volumetric operation with glass instruments: Calibration, handling repeatability, and the maximum permissible error.	Meyer et al., 2010	[79]
Comparison of two different approaches in the uncertainty calculation of gravimetric volume calibration: mainstream GUM and Monte Carlo method.	Batista et al., 2009	[80]
Ranking of the contributions to the uncertainty of titrimetric results.	Wampfler and Rösslein, 2009	[81]
Volume calibration of 1000 μl micropipettes. Inter-laboratory comparison between six national metrology institutes.	Batista et al., 2008	[82]
Primer on weighing uncertainties in radionuclidic metrology.	Collé, 2008	[83]
Measurement and uncertainty evaluation of nanofluid particle concentration using volumetric flask method.	Kostic et al., 2006	[84]
Detailed analysis of relevant uncertainty sources with two different procedures for evaluating the uncertainty identified: one of them relies on the prescribed tolerance while the other is based on the experimental estimation of the actual performance in the user's hand. The uncertainty budget for each of these two approaches is evaluated, analyzed and illustrated with a numerical example.	Kadis, 2004	[85]
Sources for both the gravimetric and spectrophotometric pipette calibration methods.	Clark and Shull, 2003	[86]
Sampling variance of ultra-dilute solutions.	Efstathiou, 2000	[87]
Experimental study using gravimetry in order to measure the variances observed in aliquot volumes delivered by graduated burettes operating with various flow-rates and surface tensions and with the burette tip immersed and not immersed in the receiving liquid.	Schwartz, 1990	[88]
Statistical methodology required for rigorous calibration of devices that are designed to deliver a fixed aliquot volume without having to read volume graduations lines.	Schwartz, 1989	[89]

Content	Reference	Ref.
Minimizing relative error in the preparation of standard solutions by judicious choice of volumetric glassware.	Lam and Isenhour, 1980	[90]
Practical guide to estimates of uncertainty of the calibration of balances.	Anonymous	[91]
Analytical balance		
Calculating measurement uncertainty of the "conventional value of the result of weighing in air".	Flicker and Tran, 2016	[92]
Weighing uncertainties in quantitative source preparation for radionuclide metrology.	Lourenço and Bobin, 2015	[93]
Influencing factors in uncertainty measurement that affect mass and volume determination. Technical specification of an analytical balance: readability, repeatability, linearity, off-center loading and hysteresis and for volumetric glassware: repeatability, readability, temperature coefficient of sensitivity, temperature scattering, meniscus reading and environmental conditions (temperature and humidity).	Rahman et al., 2015	[77]
Procedure for evaluating the uncertainty of mass measurements when using electronic balances based on the internal quality-control routine, the calibration process, the specification data sheet, and the considered weighing scenario.	González and Herrador, 2007	[9]
Influence factors that affect in uncertainty measurement of a mass determination. Technical specifications of a balance: Readability, repeatability, non-linearity, sensitivity tolerance, temperature coefficient of sensitivity and effects of environmental factors such as: air humidity, air pressure and air buoyancy.	Salahinejad and Aflaki, 2007	[94]
The influence of atmospheric pressure, air temperature, and relative air humidity on weighing results was determined in a long-term experiment.	Pozivil et al., 2006	[95]
The uncertainty evaluation of mass measurements when using "in-house" calibrated analytical balances is revisited according to the GUM.	González et al., 2005	[96]
Good practice guide is intended as a useful reference for those involved in the practical measurement of mass and weight.	Davidson et al., 2004	[97]
Influence factors which are part of the combined measurement uncertainty of a mass determination and their interplay, namely the technical specifications of the balance (repeatability, nonlinearity, sensitivity tolerance, and temperature coefficient of the sensitivity) and the effect of air buoyancy.	Reichmuth et al., 2004	[98]
A new method to correct for the largest systematic influence in mass determination – air buoyancy. Full description of the most relevant influence parameters and the combined measurement uncertainty is evaluated according to the ISO–GUM approach.	Wunderli et al., 2003	[99]
Evaluation of methods for estimating the uncertainty of electronic balance measurements. Terminology used to describe measurement quality, i.e., "accuracy," "precision," "linearity," "hysteresis," "measurement uncertainty" (MU), and the various contributors to MU, and will discuss the advantages and limitations of various methods for estimating MU.	Clark and Shull, 2001	[100]
The influence of variations in atmospheric pressure on the uncertainty budget of weighing results.	Kehl et al., 2000	[101]
Comprehensive mass metrology: A survey of the current problems surrounding mass determination that is comprehensive but does not purport to be complete.	Kochsiek and Gläser, 2000	[102]
Calibration curve		
Common mistakes in evaluating the uncertainty when pursuing that strategy, as revealed in current chromatographic literature.	Kadis, 2017	[13]

Content	Reference	Ref.
The quality coefficient as performance assessment parameter of straight line calibration curves in relationship with the number of calibration points.	de Beer et al., 2012	[103]
Comparison in the evaluation of measurement uncertainty in analytical chemistry testing between the use of quality control data and a regression analysis.	Sousa et al., 2012	[104]
Application of various methodologies concerning the estimation of the standard uncertainty of a calibration curve used for the determination of sulfur mass concentration in fuels.	Theodorou et al., 2012	[105]
The evaluation of uncertainty for linear calibration curves generation in analytical laboratories.	Nezhikhovskiy et al., 2006	[106]
Uncertainty functions: a way of summarizing or specifying the behavior of analytical systems.	Thompson, 2011	[107]
Calibration in atomic spectrometry: a tutorial review dealing with quality criteria, weighting procedures and possible curvatures.	Mermer, 2010	[108]
Critical review on the usual procedures for testing the accuracy of analytical methods.	Kemény et al., 2009	[109]
Three different techniques for fitting straight lines to experimental data and evaluation of uncertainty: (i) traditional fitting by least-squares, (ii) a Bayesian linear-regression analysis and (iii) an analysis according to the propagation of probability density functions attributed to the points measured.	Willink, 2008	[110]
New method for propagating uncertainty, based on interpolation theory, to solve the problem in linear interpolating equations. The method is extended to nonlinear equations, and to over-determined linear or nonlinear equations fitted by least-squares methods.	White and Saunders, 2007	[111]
Propous theory to calculate the confidence intervals of calibration lines in the above situations. Analyses made up of sample weighing, dilution, High Performance Liquid Chromatography measurement and calibration with the linear least-squares fitting are taken as examples.	Hayashi and Matsuda, 2006	[112]
Commonly used expression for the standard error of a result obtained from a straight line calibration is extended to a quadratic calibration, and the case where weighted regression is necessary.	Hibbert, 2006	[113]
The use of Crystal-Ball is illustrated with two working examples dealing with specification models of non-linear features and with correlated variables (such as the slope and intercept of calibration straight lines).	González et al., 2005	[114–115]
Introduction of a novel approach on actual calibration data for the determination of Pb by inductively coupled plasma-atomic emission spectroscopy. The improved calibration uncertainty was verified from independent measurements of the same sample by demonstrating statistical control of analytical results and the absence of bias.	Heydorn and Anglov, 2002	[116]
Evaluation of measurement uncertainty for analytical procedures using a linear calibration function: the uncertainty deduced from repeated observations of the sample vs. the uncertainty deduced from the standard residual deviation of the regression.	Brüggemann and Wennrich, 2002	[117]
Evaluation of the most conflicting points concerning linear regression. Confidence bands and a discussion about the use of a line through the origin are also included. In addition, the simplest expressions for expressing parameters to the appropriate significant figures from built-in calculator programs are also provided.	Giordano, 1999	[118]

Content	Reference	Ref.
Strategy for the validation of the calibration procedure in atomic absorption spectrometry. In order to accomplish this, the suitability of different experimental designs and statistical tests, to trace outliers, to examine the behavior of the variance and to detect a lack-of-fit, was evaluated. Parametric as well as randomization tests were considered.	Penninckx et al., 1996	[119]
The "precision pattern space" is introduced in order to find the general expression for the law of random error propagation. A new approach to the determination of the optimum working range in spectrophotometric procedures has been developed. The method involves the use of the calibration curve and the application of the Laplacian operator to concentration.	Asuero et al., 1988	[120]

Table 6. Selected papers about the estimation of uncertainty in volumetric glassware, analytical balance and calibration curves.

Content	Reference	Ref.
Gravimetry		
Evaluation of purity with its uncertainty value in high purity lead stick by conventional and electro-gravimetric methods.	Singh et al., 2013	[121]
The determination of barium by the gravimetric method, in which the precipitation of $BaSO_4$ was formed and weighed, coupled with instrumental measurement of trace constituents was studied. Sources of uncertainty were assessed thoroughly.	Li et al., 2002	[122]
Titrimetry		
Measurement procedure for precisely determining hypochlorite in commercial bleaches, with established traceability and full description of its uncertainty using automatic potentiometric titration.	Barbieri Gonzaga and Rodrigues Cordeiro, 2014	[123]
Calculation of measurement uncertainty in the determination of the concentration of a freshly prepared solution of sodium hydroxide using potassium hydrogen phthalate as the primary standard.	Mettler Toledo, 2014	[124]
Evaluation of measurement uncertainty components associated with the results of complexometric determination of calcium in ceramic raw materials using EDTA.	Basak and Kundu, 2013	[125]
Target measurement uncertainty as a tool for validation of uncertainties estimated by different approaches: determination of total hardness in drinking and natural waters.	Calisto et al., 2013	[126]
An easy uncertainty evaluation of the chemical oxygen demand titrimetric analysis in correlation with quality control and validation data.	Amanatidou et al., 2012	[127]
Uncertainty estimation in measurement of pKa values in nonaqueous media: a case study on basicity scale in acetonitrile medium.	Sooväli et al., 2006	[128]
Uncertainty of chemical oxygen demand determination in wastewater samples. The major sources of uncertainty of the result of measurement were identified as the purity of reagents, volumetric operations, gravimetric operations, bias, and the repeatability of the method.	Drolc et al., 2003	[129]
Analytical procedure for the determination of the concentration of hydrochloric acid by titration against a standardized sodium hydroxide solution. The expanded uncertainty of the final result is expressed, endeavoring, in particular	Pueyo and Vilalta, 1996	[130]

Content	Reference	Ref.
to evaluate covariances and to take into account the chemical behavior of the specific reagent.		
Potentiometry: Ion Selective Electrode		
Uncertainty evaluation in the chloroquine phosphate potentiometric titration: Application of three different approaches: The famous error-budget approach, the analytical method committee top-down and the last method chosen was the one proposed by Barwick and Ellison.	Rodomonte et al., 2006	[131]
Procedure to estimate the uncertainty of measurement applied to the fluoride determination of waters and wastewaters matrices by selective electrode potentiometry based on Eurachem Guide. The major sources of uncertainty were identified as the calibration standard solutions, fluoride concentration obtained by potential interpolation of the regression line and the precision.	Sousa and Trancoso, 2005	[132]
Estimation of uncertainty in measurement of the pK_a of a weak acid by potentiometric titration. The procedure is based on the ISO GUM.	Koort et al., 2004	[133]
Amperometry		
Tutorial review on measurement uncertainty estimation in amperometric sensors.	Helm et al., 2010	[134]
Electron probe microanalysis		
Case study of ISO GUM-based estimation of measurement uncertainty of quantitative surface elemental analysis by electron probe microanalysis.	Virro et al., 2008	[135]
Ultraviolet Spectrophotometry		
Procedure to estimate measurement uncertainty of a validated UV spectrophotometric method for quantification of desloratadine in tablet formulation.	Takano et al., 2017	[136]
Uncertainty in spectrophotometric analysis – "Error propagation break up", a novel statistical method for uncertainty management. For the assessment of the computations, different approaches are discussed, such as the contribution to the Combined Standard Uncertainty of the reproducibility, the repeatability, the total bias, the calibration curve, and the type of the measurand.	Amanatidou et al., 2011	[137]
Eevaluation of the uncertainty and metrological reliability of material concentration measurement considering sample preparation and chemical–physical transformation of spectrometric analysis.	Dobiliene et al., 2010	[138]
Uncertainty in modern spectrophotometers: An up-to-date view of UV–vis molecular absorption instruments and measurements.	Galbán et al., 2007	[139]
Overview of the most important uncertainty sources that affect analytical UV–Vis spectrophotometric measurements. Altogether, eight uncertainty sources are discussed that are expected to have influence in chemical analysis.	Sooväli et al., 2006	[128]
Procedure for estimation of measurement uncertainty of photometric analyis based on the ISO GUM method. Two variations of the procedure, for the calibration graph and the standard addition method, are discussed.	Traks et al., 2005	[140]
Evaluation of the uncertainty of measurement in the determination of manganese by spectrophotometric analysis. The standard uncertainty is evaluated for each input quantity. These are then appropriately combined to get the combined uncertainty of measurement.	Ramachandran and Rashmi, 1999	[141]
X-Ray Fluorescence Spectrometry		
Evaluation of uncertainty in the energy dispersive X-ray fluorescence determination of platinum in alumina.	Remya Devi et al., 2015	[142]

Content	Reference	Ref.
Uncertainty measurement evaluation of wavelength dispersive and energy dispersive X-ray fluorescence techniques for the Si and Utotal determination in U_3Si_2 nuclear fuel.	Scapin et al., 2011	[143]
Uncertainty calculations for the measurement of in vivo bone lead by X-ray fluorescence	O'Meara and Fleming, 2009	[144]
Effect of the sample matrix on measurement uncertainty in X-ray fluorescence analysis.	Morgenstern et al., 2005	[145]
Atomic Absorption Spectrometry		
Determination and uncertainty analysis of inorganic arsenic in husked rice by solid phase extraction and atomic absorption spectrometry with hydride generation.	Saxena et al., 2017	[146]
Optimization and measurement uncertainty estimation of hydride generation–cryogenic trapping–gas chromatography–cold vapor atomic fluorescence spectrometry for the determination of methylmercury in seawater.	Živković et al., 2017	[147]
Approach for the estimate of the uncertainty in measurement considering the individual sources related to the different steps of the method under evaluation as well as the uncertainties estimated from the validation data for the determination of mercury in seafood by using thermal decomposition/amalgamation atomic absorption spectrometry.	Torres et al., 2015	[148]
Methodology of evaluating the uncertainty of measurement of chemical composition using atomic absorption spectrometry.	Mahajan et al., 2012	[149]
Comparison of ISO-GUM and Monte Carlo methods for the evaluation of measurement uncertainty: Application to direct cadmium measurement in water by graphite furnace atomic absorption spectrometry.	Theodorou et al., 2011	[150]
Evaluation of measurement uncertainties for the determination of total metal content in soils by atomic absorption spectrometry.	Alves et al., 2009	[151]
Uncertainty statement of a mercury speciation analytical method using the relationships fixed by GUM (Guide to the Expression of Uncertainty in Measurement).	Jokai and Fodor, 2009	[152]
UV–Vis spectrophotometric and flame atomic absorption spectrometric analysis for iron determination in a pharmaceutical product were compared in terms of uncertainty budgets.	Jürgens et al., 2007	[153]
How to validate the calibration function is dealt with in detail using as an example based on measurements obtained for nickel determination by flame atomic absorption spectrometry. Assessing uncertainties related to linear calibration curves is also discussed.	Chui, 2007	[154]
Three approaches are compared for the evaluation of the combined uncertainty in the determination of mercury in aquatic sediments by an aqua regia extraction procedure.	Guevara-Riba et al., 2006	[155]
Full validation of a cold vapor atomic absorption spectrometry method for mercury determination in fishery products.	Haouet et al., 2006	[156]
Uncertainty of atomic absorption spectrometer.	Hirano et al., 2005	[157]
Estimate of uncertainty of measurement from a single-laboratory validation study: application to the determination of lead in blood.	Patriarca et al., 2004	[158]
Total uncertainty budget calculation for the determination of mercury in incineration ash (BCR 176R) by atomic fluorescence spectrometry.	Tirez et al., 2002	[159]

Content	Reference	Ref.
Uncertainty of measurement of the analysis of lead in blood by graphite furnace atomic absorption spectrometry calibrating with a commercial available standard.	O 'Donnell, 2000	[160]
The major sources of uncertainty of a method for determination of Pb in whole blood by atomic absorption spectrometry. The combined uncertainty was compared to the experimentally determined variation and a satisfactory agreement was found, indicating that no significant sources of uncertainty have been overlooked and that the method is in a state of statistical control.	Kristiansen et al., 1996	[161]
Nuclear Magnetic Resonance Spectroscopy		
Uncertainty budget for the results of measurements of purity of the agrochemical glyphosate using ^{1}H and ^{31}P quantitative nuclear magnetic resonance spectroscopy. The budget combines intralaboratory precision from repeated independent measurements of a batch, and other Type A and Type B effects.	Al-Deen et al., 2004	[162]
Inductively Coupled Plasma		
Results of prominent technologies of inductively coupled plasma mass spectrometry, for determination of chloride-isotope ratios ($^{35}Cl/^{37}Cl$) and inductively coupled plasma optical emission spectrometry for determination of sodium, were evaluated in terms of the true level of uncertainty and revealed a genuine problem for science that was not addressed in VIM3 and QUAM.	Andersen et al., 2016	[163]
Application of the GUM approach to estimate the measurement results uncertainty for the quantitative determination of Al, Ba, Fe, Mg, Mn, Pb, Sr. and Zn from document paper samples using Inductively Coupled Plasma Mass Spectrometer. The measurement uncertainty estimation was done based on identifying, quantifying and combining all the associated sources of uncertainty separately.	Tanase et al., 2015	[164]
Development, validation, and evaluation of measurement uncertainty of a method for quantitative determination of essential and nonessential elements in medicinal plants and their aqueous extracts by using inductively coupled plasma optical emission spectrometry.	Senila et al., 2014	[165]
Uncertainty budget for multi-elemental analysis of plant nutrients in conifer foliar material using inductively coupled plasma atomic emission spectrometry.	Ohlsson, 2012	[166]
Method for simultaneous inductively coupled plasma mass spectrometer determination of 13 elements in three types of honey from Poland. The method was validated, and the uncertainty budget was set up.	Chudzinska et al., 2012	[167]
Evaluation of the combined measurement uncertainty in isotope dilution by a multi-collector inductively coupled plasma mass spectrometer and the use of high-purity reference materials.	Fortunato and Wunderli, 2003	[168]
Validation of the determination of lead in whole blood by inductively coupled plasma mass spectrometer. Uncertainty of the centroid of the calibration graph was preferred to the evaluation of the linearity with ANOVA to validate the calibration procedure.	Bonnefoy et al., 2002	[169]
The measurement uncertainty associated with the determination of Ni in aqueous samples by inductively coupled plasma mass spectrometer has been calculated using a cause-and-effect approach. A cause-and-effect diagram was constructed to aid in the identification of the sources of uncertainty associated with the method.	Barwick et al., 1999	[170]

Content	Reference	Ref.
Mass spectrometry		
The application of the GUM to calculate standard uncertainties for routine uranium isotope mass spectrometry measurements for nuclear safeguards and nuclear metrology.	Bürguer et al., 2010	[171]
Chromatography		
Study to estimate a reasonable uncertainty for the measurement of the identified measurand, which is the mass concentration of ethanol, methanol, acetone, and isopropanol determined through dual capillary column headspace gas chromatograph (GC): GC calibration adjustment slope, GC analytical, and certified reference material.	Hwang et al., 2017	[172]
Development, validation and different approaches for the measurement uncertainty of a multi-class veterinary drugs residues liquid chromatography-mass spectrometry method for feeds.	Valese et al., 2017	[173]
Critical challenges regarding the validation of a quantitative multi-residue method for pharmaceuticals in wastewater making use of modern solid phase extraction-liquid chromatography-orbitrap high-resolution mass spectrometry. Particular attention is given to study in detail response linearity, to realistically estimate detection limits, and to express the measurement precision of the analyte concentration, obtained by external calibration.	Vergeynst et al., 2017	[174]
Validation and uncertainties evaluation of an isotope dilution-solid phase extraction-liquid chromatography–tandem mass spectrometry for the quantification of drug residues in surface waters.	Brieudes et al., 2016	[175]
Rapid determination of residues of pesticides in honey by gas chromatography–electron capture detector and gas chromatography–tandem mass spectrometry: Method validation and estimation of measurement uncertainty according to document No. SANCO/12571/2013.	Paoloni et al., 2016	[176]
A fast and simple liquid chromatography–tandem mass spectrometry method for detecting pyrrolizidine alkaloids in honey with full validation and measurement uncertainty.	Valese et al., 2016	[177]
Comparison of different methods to estimate the uncertainty in composition measurement by chromatography: two of them (guide to the expression of uncertainty in measurement method and prediction method) were compared with the Monte Carlo method.	Ariza et al., 2015	[178]
The role of uncertainty regarding the results of screening immunoassays in blood establishments.	Pereira et al., 2015	[179, 180]
Determination of polybrominated diphenyl ethers in water by a simple dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry method. Validation parameters, including the calculation of the expanded uncertainty associated to the results in the range of quantification is included.	Santos et al., 2015	[181]
Establishing measurement of uncertainty for simultaneous bio-analytical determination of L-Carnitine and Metformin in human plasma by liquid chromatography–tandem mass spectrometry.	Terish Bino et al., 2015	[182]
Contribution of each stage in the developed procedure on the uncertainty measurement of the determination of volatile aromatic hydrocarbons in surface and underground water. The uncertainty sources were identified and illustrated in an effect diagram.	Pavlova et al., 2014	[183]

Content	Reference	Ref.
Evaluation of the sources of uncertainty in the determination of repaglinide in human plasma using liquid chromatography–tandem mass spectrometry.	Li et al., 2013	[184]
Measurement uncertainty of food carotenoid determination. The ISO guide was interpreted for analytical chemistry by EURACHEM. Measurement uncertainty was estimated based on laboratory validation data, including precision and method performance studies, and also, based on laboratory participation in proficiency tests.	Dias et al., 2012	[185]
Comparison of measurement uncertainty component estimations for three methods using the high-performance liquid chromatography techniques: determination of the type and content of aromatic hydrocarbons in diesel fuels and petroleum distillates by normal phase high-performance liquid chromatography, determination of nitrates in water samples by ion chromatography, and determination of molecular weights of polystyrene by size exclusion chromatography technique.	Tomić et al., 2012	[186]
The estimation and use of measurement uncertainty for a drug substance test procedure validated according to USP <1225>.	Weitzel, 2012	[187]
Estimation of the global uncertainty associated to the determination of pentachlorophenol in aqueous samples, by gas chromatography with mass spectrometric detection, after solid phase microextraction.	Brás et al., 2011	[188]
A high-performance technique that was originally developed for inductively coupled plasma optical emission spectrometry has been successfully translated to ion chromatography to enable analyses with extremely low uncertainty (0.2% Relative Expanded Uncertainty).	Brennan et al., 2011	[189]
Estimating the uncertainty related to GC-MS analysis of organo-chlorinated pesticides from water.	Pana et al., 2011	[190]
Development of a model system of uncertainty evaluations for multiple measurements by isotope dilution mass spectrometry: determination of folic acid in infant formula.	Kim et al., 2010	[191]
Basic terms, sources of uncertainty, and methods of calculating the combined uncertainty.	Konieczka and Namieśnik, 2010	[192]
Evaluation of uncertainty of measurement from method validation data: An application to the simultaneous determination of retinol and -tocopherol in human serum by high performance liquid chromatography.	Semeraro et al., 2009	[193]
Estimating the measurement uncertainty in forensic breath-alcohol analysis.	Gullberg, 2006	[194]
Uncertainty budget for final assay of a pharmaceutical product based on reverse phase high performance liquid chromatography.	Anglov et al., 2003	[195]
Analytical method to verify the accuracy of the natural abundance butyltin standard concentrations that are needed for their subsequent use in the reverse spike isotope dilution quantitation of enriched species-specific spikes. A full combined uncertainty calculation, accounting for all possible sources of uncertainty in the measurement process.	Yang et al., 2002	[196]
Propagation of uncertainty in high-performance liquid chromatography with UV–VIS detection.	Hibbert et al., 2001	[197]

Table 7. Selected papers on evaluation of the measurement uncertainty in classical and instrumental techniques.

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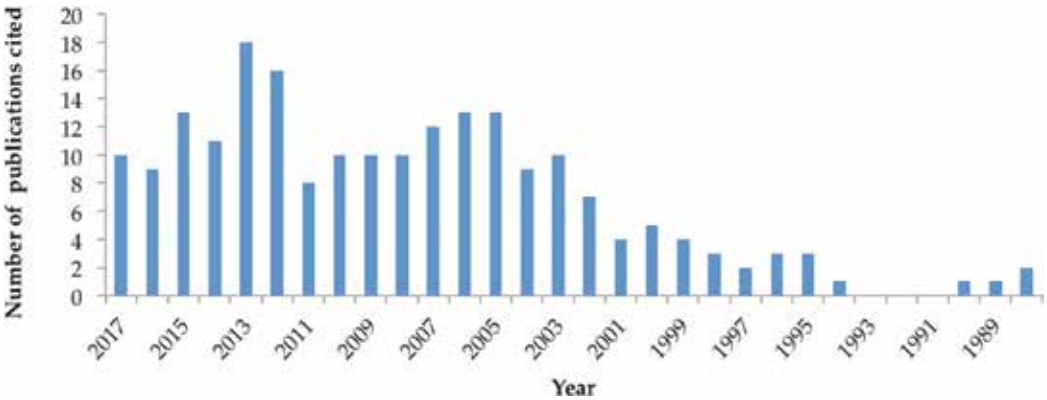

Figure 2. Number of publications cited per year.

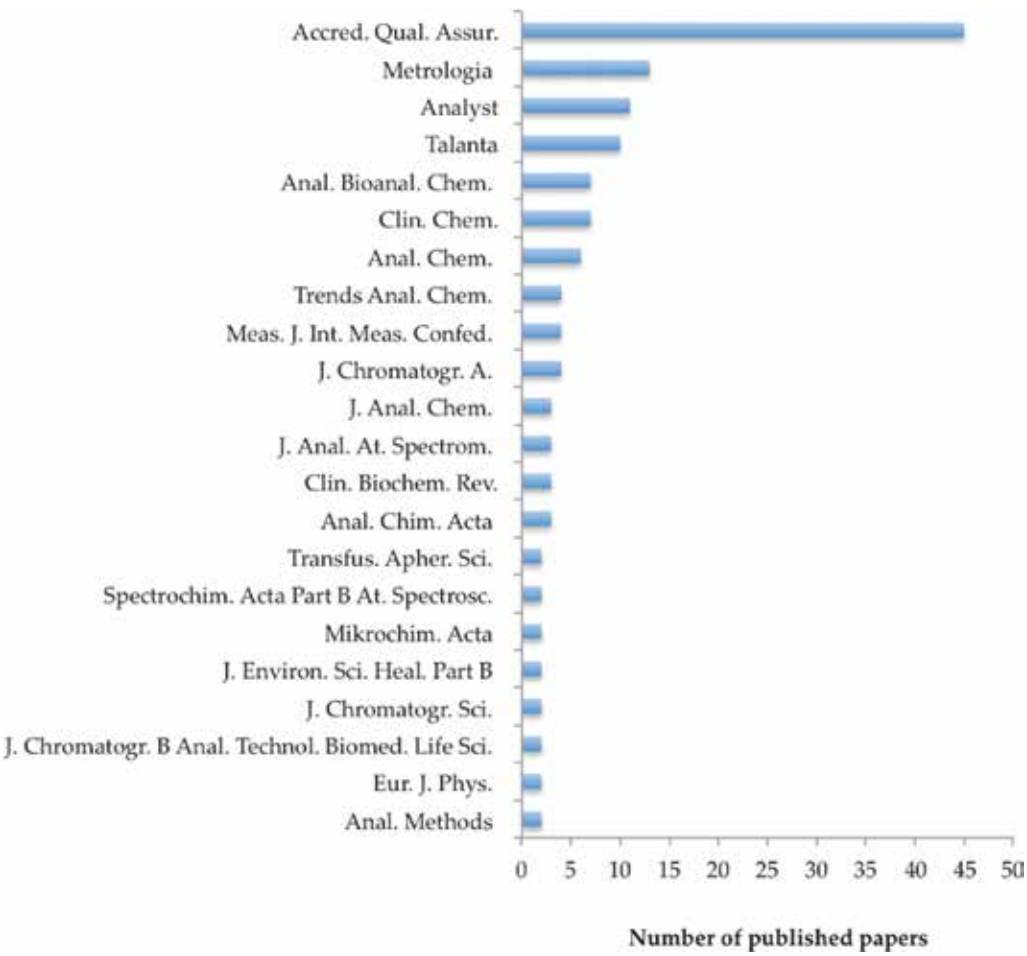

Figure 3. Number of papers cited by journals.

4. Final comments

Uncertainty is a measure of the quality of a measurement. It is of vital importance in many sectors of analytical chemistry to introduce quality control and quality assurance in production, complying with and enforcing laws and regulations; calibrating standards and instruments or developing and comparing international and national reference standards among others.

One of the best-known approaches to estimate the uncertainty of analytical procedures is the ISO/GUM. However, from an analytical viewpoint, this approach is sometimes tedious, time-consuming and unrealistic. One way to overcome these limitations is the procedure for evaluating uncertainty of analytical assays in routine analysis using the GUM approach together with the data from in-house validation based on the cause and effect diagram coming from the analytical specification function. Expressions to calculate the different contributions of uncertainty have to be carefully adapted in order to avoid double counting. The procedure is illustrated with a case study on fluorimetric determination of quinine in tonic water showing that it is very suitable for evaluating the uncertainty of the analyte content of future samples in routine analysis.

Finally, a summary including modern reviews on the estimation of measurement uncertainty of analytical assays by GUM is outlined in tabular form, which can be a useful guide for those interested in the subject. Moreover, selected application ranging from volumetric glassware, analytical balance, calibration curves, as well as the evaluation of the measurement uncertainty in classical and instrumental techniques in a wide variety of fields are given. Graphs on the number of references cited (over 200) per year and the number of papers by most cited journals are also included.

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