COURSE NOTES
A model for calculating measurement uncertainty in medical laboratories

Model pentru calcularea incertitudinii de măsurare în laboratoarele medicale

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Abstract

Introduction: *All medical laboratories that require recognition for competency assessment have to estimate the uncertainty of measurement of assay test results "where relevant and possible" (ISO 15189:2007 Medical laboratories - Particular requirements for quality and competence). The repeated quantitative examination of an analyte with the same method will offer more or less different results. This is happening because the outcome of an assay depends not only upon the analyte itself, but also upon few error factors that could yield doubts about the obtained result. The mathematical, quantitative expression of this doubt is known as uncertainty of measurement (UM).* ***Methods:*** *It is the responsibility of each medical laboratory to identify all error sources that can be quantified and converted in standard deviations that could be used to estimate the type A or B of uncertainty. In the case of Romanian medical laboratories, the European Accreditation (EA) accepted as reference documents for UM estimation the Guide to the Expression of Uncertainty in Measurement (GUM) and Romanian Standard SR ENV 13005.* ***Discussion and conclusion:*** *In this paper, authors present and discuss the modalities of UM estimation in two different situations: when the used reference materials (calibrators) are or are not traceable to certified reference materials (CRM). Complete and informative UM reporting can only lead to better decisions in healthcare.*

Keywords: *precision, accuracy, uncertainty of measurement, calibrator.*

Rezumat

Introducere. *Toate laboratoarele medicale care doresc recunoaştere de competenţă trebuie să-şi estimeze incertitudinea de măsurare acolo unde este "relevant şi posibil" (SR EN ISO 15189, Standard Român, Laboratoare medicale, Cerinţe particulare pentru calitate şi competenţă, 2007). În cazul unor măsurari repetate ale unui analit obţinem rezultate diferite, mai mult sau mai puţin apropiate între ele, deşi valoarea analitului*

este aceeaşi. Valoarea unui rezultat măsurat nu depinde numai de valoarea însăşi, ci şi de o serie de factori de eroare, care aduc o neîncredere, un dubiu asupra rezultatului obţinut. Exprimarea matematică, cantitativă a acestei neîncrederi se numeşte incertitudine de măsurare (UM). ***Material şi metodă.*** *Este responsabilitatea fiecărui laborator să-şi indentifice toate sursele de eroare care pot fi cuantificate şi convertite în deviaţii standard pe baza cărora să-şi estimeze UM de tip A şi de tip B. Pentru laboratoarele medicale din România, European Acreditation (EA) recunoaşte ca documente de bază pentru estimarea UM, Guide to the Expression of Uncertainty in Measurement (GUM) şi Standardul Român SR ENV 13005 (Ghid pentru exprimarea incertitudinii de măsurare).* ***Discuţii şi concluzii.*** *Autorii prezintă modalităţile de estimare a UM în laboratoarele medicale din ţara noastră când calibratorii utilizaţi sunt trasabili sau nu la materiale de referinţă certificate (MRC). Raportarea corectă şi completă a UM influenţează decizia terapeutică.*

Cuvinte-cheie: *precizie, acurateţe, incertitudine de măsurare, calibrator.*

All medical laboratories that require recognition for competency assessment have to estimate the uncertainty of measurement of assay test results "where relevant and possible" (1).

The term "uncertainty" means a doubt and "uncertainty of measurement" (UM) is a doubt on the validity of outcome measurements. UM is only applicable to results of numerical quantitative measurement.

According to GUM "the result of a measurement is only an approximation or estimate of the value of the measurand and thus is complete only when accompanied by a statement of the uncertainty of that estimate" (2). Indeed, due to measurement uncertainty, a 'true value' of measurement can never be known.

There are several definitions of the UM:

- "estimation of the range of values within which the true value of a measurand lies" (2);
- "parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used" (3).

From daily practice it is well known that when a measurement is repeated n times we obtain n different results, although the analyte is the same. This happens because the results of a measurement are depending not only upon the analyte itself, but also upon a number of error factors that could yield doubts about the estimate.

It is important not to confuse the terms „error of measurement" and "uncertainty of measurement" (2, 3). An error of measurement is the difference between the measured value and the "true value" of the analyte being measured. As such, the error is a single value that can be applied as a correction to the result. The UM is a range of values and this range cannot be used to correct a measurement's result (4).

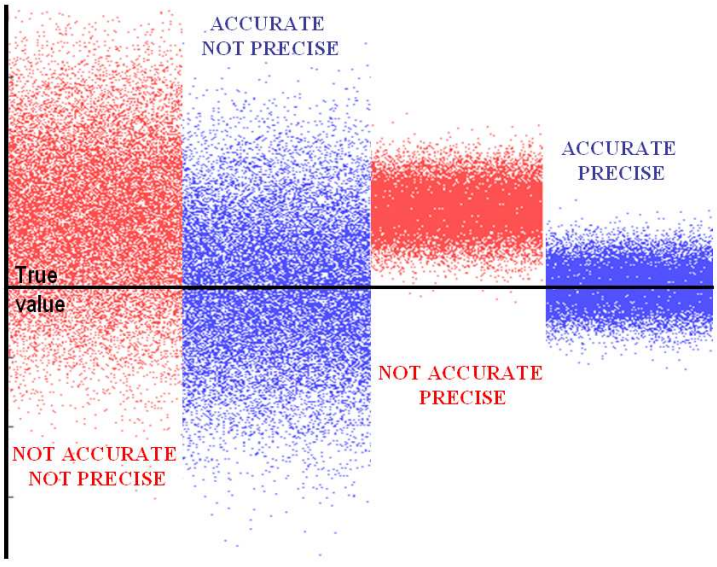
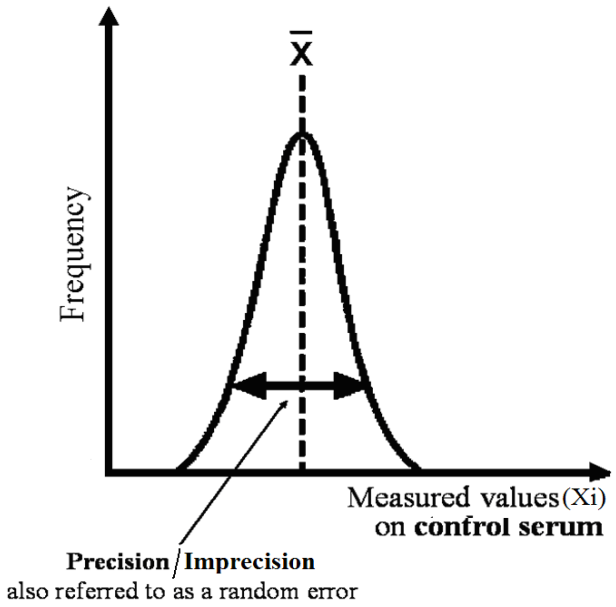


Figure 1. Precision and accuracy of measurements (modified after Mark Martinec http://www.ijs.si/time/).



$$\overline{X} = \frac{\sum_{x=1}^{n} x_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (Xi - \overline{X})^2}{n-1}}$$

$$CV = \frac{SD}{\overline{X}} \bullet 100 \text{ , where}$$

$\overline{X}$ = average of measured values
Xi = individual measured values
SD = standard deviation
CV = coefficient of variation
n = number of measurements

Figure 2. Precision of measurements on control serum.
Results obtained by repeated measurements on a control material have a Gaussian distribution.

The UM is the number after "±" sign and it is expressed as "standard deviation (or a given multiple of it), or the half-width of an interval having a stated level of confidence" (2).

Uncertainty of measurement comprises, in general, many components grouped in 2 types (A and B) derived from method for estimation of their numerical value (2, 5, 6).

Type A uncertainty is obtained by calculation from a series of repeated measurements, using statistical methods. Type A uncertainty comprises random errors which arise from random effects. Random errors cannot be eliminated but the uncertainty due to their effect may be reduced by increasing the number of measurements and applying statistical analysis.

Type B uncertainty appears by means other than those used for a Type A evaluation. For example, by using data from outside sources: calibration certificates, manufacturers' specifications, previous measurement data, experience with the behavior of the instruments, intercomparison scheme and all other relevant information. Type B uncertainty encompasses systematic errors.

Each component of uncertainty is represented by an estimated standard deviation named standard uncertainty (Ui) equal to the positive square root of the estimated variance.

The performance of quantitative tests from medical laboratory is attested by two components: precision and accuracy of measurements (*Figure 1*) (7). These two components should be taken into account for a reasonable estimation of measurement uncertainty in medical laboratories.

Precision is characterized by the dispersion of values obtained by repeated measurements of an analyte and it is expressed by standard deviation (SD) and coefficient of variation (CV) (*Figure 2*).

Precision of measurements is evaluated by type A uncertainty (8).

Type A uncertainty is based on a statistical analysis of n different values obtained from repeated measurements of an analyte using the same method.

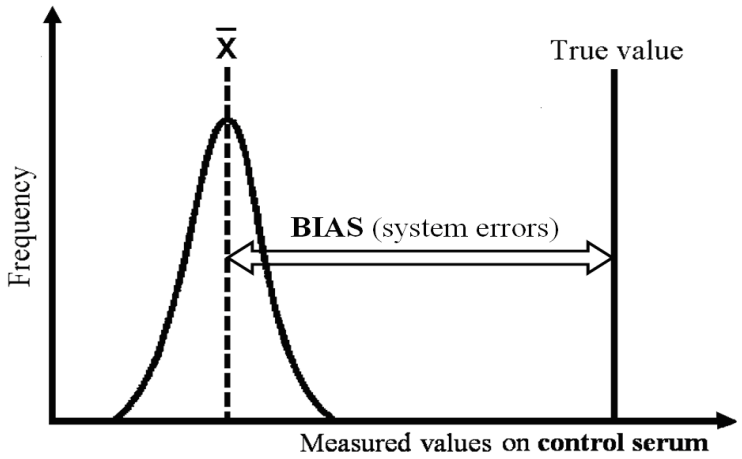


Figure 3. The accuracy of the measurement refers to closeness of the agreement between the result of a measurand and a true value.

The mean is determined by adding a group of measured values, then dividing the total by the number of measurements in the group as equation [1]:

$$\overline{X} = \frac{x_1 + x_2 + ... + x_i + ... + x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n} \quad [1],$$

where:

$\overline{X}$ = mean of the measured values,

Xi = individual measurements,

n = number of the values Xi in the group.

Then the standard deviation is determined by equation [2].

This standard deviation (SD) represented by a statistically estimate (experimental standard deviation) constitutes type A uncertainty.

There are many possibilities for assessing type A uncertainty. Type A uncertainty is based on repeated measurements of the same analyte over a long period of time. For that reason, medical laboratories estimate type A measurement uncertainty by using control material (in which the analytes are stable for a long period of time). Control material is homologue to patient sample. Control material is measured by the same measurement system, with the same calibrator and reagents as patient sample. Therefore, by extrapolation, the precision of measurements using the control material is identical with the precision of measurements using patient sample in which the analytes have only short-term stability.

Accuracy represents the difference between the result (the average of results) obtained by measurement and the true value of a measurand which is expressed by Bias (*Figure 3*).

Accuracy of measurements is evaluated by type B uncertainty. Type B uncertainty is determined by other means than type A uncertainty, namely non statistical methods from *a priori* data:

- uncertainty of the value assigned to the calibrators
- the data from external control (intercomparisons)
- traceable certificate report of equipment
- data from validation process (2, 9).

The estimation of the UM increases the reliability of the results obtained in medical laboratories. For a higher credibility, every single measured result must be accompanied by UM.

The component standard uncertainties are combined according to the law of propagation of uncertainty to produce an overall value

Equation [2]:

$$SD = \sqrt{\frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + ... + (x_n - \overline{x})^2}{n-1}} = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$$

, where

X_i = individual values obtained from repeated measurements

$\overline{X}$ = mean of the measured values

n = number of measurements (2, 6).

of uncertainty, known as the **combined standard uncertainty (UMc)**. UMc is given by the square root of the sum of the squares:

$$UM_C = \pm\sqrt{U_A^2 + U_B^2}$$ [3].

Expanded uncertainty (U) is calculated by multiplying the standard uncertainty UMc with an appropriate coverage factor k:

$U = k \bullet UMc$ [4], for a desired level of confidence of 95%, k is approximately 2.

Uncertainty reported with the result is expanded uncertainty and is then conveniently expressed as:

$Y \pm U$ [5], where:

Y = measured result,
U = expanded uncertainty.

The Guide to the expression of uncertainty in measurement (GUM) (2) was developed for estimating UM in all laboratories types. In non-medical laboratories the potential sources of uncertainty are usually readily identifiable, quantifiable and converted in standard deviations in fields such as physical and chemical measurements (e.g. electrical, materials, optics, etc). These standard deviations are used for calculation of UMc.

The Working Group for the Medical Testing Laboratory recognizes that the implementation of the uncertainty of measurement requirement offers opportunities for pathology laboratories to add value to their diagnostic services, particularly in educating users to better understand the limitations of tests, and in recognizing when clinically significant changes in patient results have or have not occurred. ISO 15189 also recognizes that the rigor of estimating uncertainty of measurement may be based on the needs of the client.

In medical testing there are many potential "uncertainties" that can significantly affect test results (for example: poor specimen collection or transport, patient related factors such as biological variation and the presence of drugs, clerical and reporting errors etc). Not all these factors can be quantified in standard deviations and therefore such factors are excluded from the estimation of uncertainty of measurement (7). It is important to identify such factors and keep them under an adequate management. In medical laboratories is difficult to strictly respect GUM regulations. However, medical laboratory measurements are strictly monitored by an internal and external control system, and therefore the data generated can be used to estimate UM.

A basic requirement of GUM is to establish a mathematical expression which has to include all the input quantities that significantly influences the test result. In medical laboratories rigorously implementation of GUM is difficult, but the above requirement of GUM must be respected.

Whatever approach is used, estimation of UM should consider the basic requirements of GUM and the following steps (2, 4):

1. complete definition (or specification) of the measurand;
2. careful consideration and understanding of each phase of the measurement process;
3. mathematical expression of the equation relating measurand (Y) and input quantities (X_i) using a functional relationship in the form $Y = f(X_1, X_2, X_3...X_n)$. Functional relationship *f* should enclose all input quantities that contribute to the final result;
4. estimation of a certain value for all input quantities in functional relationship *f* using statistical methods or other means;
5. evaluation of standard uncertainty for every quantity X_i;
6. calculation of combined standard uncertainty;
7. calculation of expanded standard uncertainty with a chosen coverage factor;
8. reporting uncertainty of measurement.

1. Defining the measurand as fully as possible in terms of designation, matrix, unit of measure, because lack of measurand definition brings a

major uncertainty to the final test result. This will yield major discrepancies between the measurement results in different laboratories for the same analyte.

Analyte is a term used to identify the substance or constituent of interest (e.g. creatinine) that is the subject of measurement. However, a substance can have a number of properties (e.g. concentration, different matrix, different unit of measure, different measurement methods), some or all of which can be utilized to quantify the substance in an appropriate measuring system. The particular quantifiable property of the analyte used in the measuring system is called the measurand (7).

For example, the analyte is creatinine and the measurand is creatinine concentration in serum, plasma or urine expressed in mg/dl or µmol/l. Therefore it is essential to define as completely as possible the quantity that is measured (i.e. the measurand) by a given procedure.

2. Establishing the measurement principle of the specified technical procedure for performing a test concerning of all reaction phases is exemplified by the principle of measurement of creatinine in a biological sample. Picric acid reacts in alkaline conditions with creatinine from analysed biological sample to form picramic acid. This is a colored product with a color intensity which is measured at a certain wave length (510 nm) as optic density directly proportional with creatinine concentration from analysed biological sample.

3. Establishing a mathematical formula that determines the functional relationship between measured quantity Y and measured quantities (Xi, input quantities upon which final test result depends). In order to convert optical density into a test result a reference material (calibrator) with a known value of creatinine (measurand) is used. The calibrator is measured in the same conditions as the analyzed biological sample. At the end of the reaction, optical density (OD) read by the equipment for reference material has the same value as the value assigned to the calibrator from the specifications of the manufacturer. This process it is called calibration and represents a "set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards" (3). Therefore, the result y of creatinine from patient sample could be calculated by rule of three:

2500 OD..... 3.6 (calibrator value)
1800 OD..... y

$$y = \frac{OD_{sample}}{OD_{calibrator}} \bullet value_{calibrator} \quad [6],$$

where OD is optical density (of calibrator, respectively to patient sample).

Figure 4 represents the calibration curve for creatinine in 2 points, respectively 0 and 3.6 mg/dl. If the calibrator value of 3.6 mg/dl includes UM expressed as range value (see the dotted lines of *Figure 4*), the optical density of final product reaction of patient sample, 1800, will lead to different results according to the calibration curve used.

Since the reference materials (calibrators, etalons and standards) are very homogenous it is admitted that value distribution is rectangular (9). Thus, calibration curve can be situated anywhere in the range marked with dotted lines, not necessarily in the middle of the range.

The constructed calibration curve could be validated and used for carrying out the measurements on the patient's samples only after is verified using the control materials of known values because a lot of error sources from used equipment used, reagents, medium conditions, laboratory technicians etc. may appear. The measurement system (equipment, reagents, calibration curve and analyst) is verified in terms of correctness by testing control materials on different levels. These control materials have predetermined values and could alert the analyst regarding the errors of measurement system.

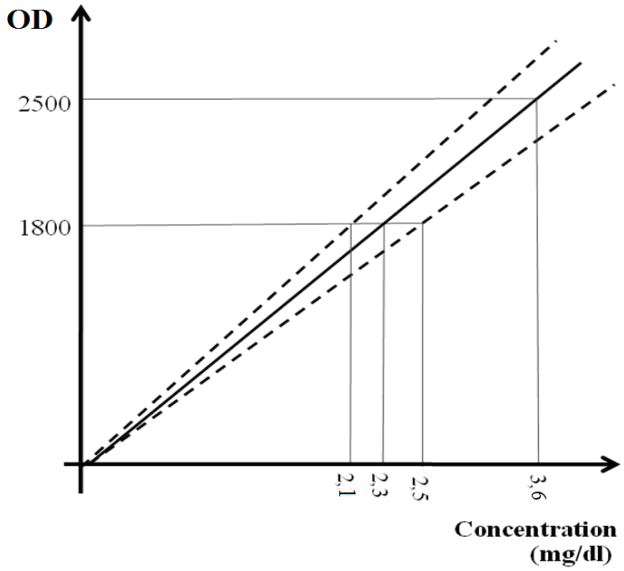


Figure 4. Calibration curve in 2 points.

The results obtained by measuring control materials are analyzed considering Westgard rules and the acceptance / rejection criteria of each laboratory. Internal control tests with control materials are similar to diagnostic tests of patient samples. Similarly with diagnostic tests in patients, which identify the health problems of the patient, the tests of internal control identify the "health problems" (errors) of the measurement system (10). Rapid detection and adequate treatment is depending upon precise and quick identification of the "health problem".

The reference materials (calibrators, etalons, standards) are considered X_i quantities upon which final test result depends, while control material is used for verifying and validating the correctness of calibration but does not have a direct influence on final result.

4. Estimation of a value for all input quantities

The result of a quantitative measurement Y is a functional relationship $f(X_i)$, where X_i represents all the factors upon which the measurement depends (X_1, X_2, X_3 ... X_n) (4, 7). All relevant sources of uncertainties that could influence the test result should be identified. Consideration should be given to all relevant factors that may contribute to the overall uncertainty of a measurement and could influence the test result. Some examples are given below:

X_1 = factors affecting pre-analytical phase cannot be usually quantified, but must be controlled by an adequate management;
X_2 = measurement procedure (method, reagents, measuring instruments, laboratory hardware etc);
X_3 = reference material (calibrator);
X_4 = factors affecting post-analytical phase;
....
X_n = others factors.

In medical laboratories, after identification of all input quantities X_i, it could be observed that the measurement procedure used (method, reagents, instruments, laboratory stuff etc.) and the reference materials (calibrators) are the **only** quantifiable factors that could be converted in standard deviations.

The best estimation for the measurement procedure used is the average of the measured values obtained in n repeated measurements (the precision) between-day and within-day measuring technique (from data of internal control).

The estimation of the value of reference material (calibrator) is offered by the manufacturer.

5. Estimation of standard uncertainty for each quantity X_i

At least 30 values of control serum on at least 30 different consecutive days were used in the measurement procedure. In this case the Xi input estimate is usually the average value. All values from the internal control have Gaussian (normal) distribution. Standard uncertainty U(xi) is the estimated standard deviation of the mean calculated by formula [2]. This standard uncertainty is estimated by statistical calculation of measurement values and therefore it is named Type A uncertainty.

When the medical laboratory uses reference materials which are not traceable to CRM, the laboratory does not know the UM of the cal-

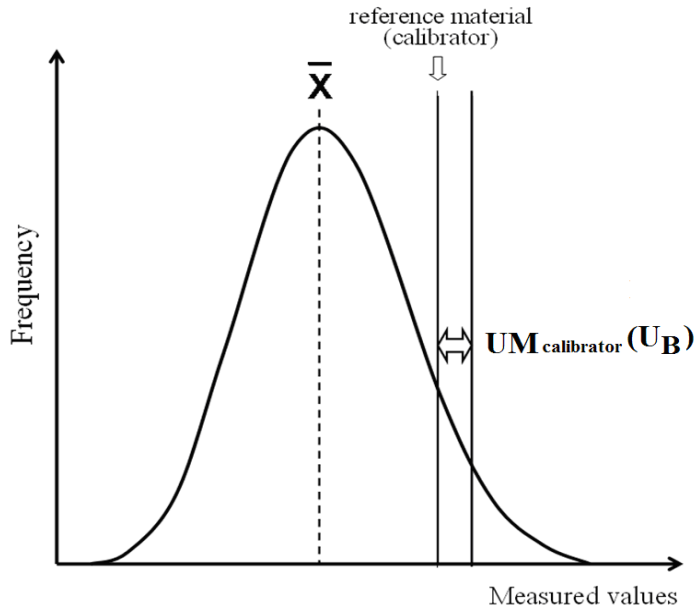


Figure 5. UM of used reference material (calibrator) represents type B uncertainty (UB) because it is taken from manufacturer specifications ($\overline{X}$ = average values measured).

ibrator although this is a measured value and is accompanied by UM. When used calibrators are traceable to certified reference materials (CRM), their values are accompanied by extended uncertainty. Uncertainty of the values assigned to the calibrator can be obtained by request from the manufacturer usually as extended uncertainty (for 95% confidence interval of the calibrator assigned value, k=2) (*Figure 5*). Combined uncertainty of the values assigned to the calibrator is used in medical laboratories, so $U_{extended}$ should be divided by 2.

Uncertainty of the values assigned to the calibrator indicates the accuracy measurement as type B uncertainty because it is taken from the manufacturer's specifications and it is not a result of statistical calculations performed by the medical laboratory (11, 12).

6. Calculation of combined standard uncertainty (UMc)

Calculation of combined standard uncertainty is done following equation [5], in which the medical laboratory has the responsibility to choose many U_{Bi}:

$$UMc = \pm\sqrt{U_A^2 + U_{B_1}^2 + ... + U_{B_n}^2} \quad [7]$$

where:

$U_A = U_{\text{measurement procedure}}$ *(from internal quality control),*

$U_{B1} = U_{\text{calibrator}}$ *(from manufacturer specifications),*

$U_{B2} = U_{Bias}$ *(from external quality control),*

$U_{\text{other factors}}$ (*from speciality literature data*) (4).

Under these conditions, equation [5] becomes equation [8].

7. Calculation of expanded standard uncertainty ($UM_{expanded}$) with a chosen coverage factor

Expanded uncertainty, usually shown by the symbol *U*, is obtained by multiplying the UMc by a coverage factor, denoted by symbol k = 2, for a level of confidence of approximately 95% (2).

$$UM_{\text{expanded}} = U = UMc \cdot k \quad [9].$$

8. Reporting uncertainty of measurement

Most laboratories have chosen until now not to state measurement uncertainty in their test reports. Instead, such information has been given only when the physicians have specifically required it. It is important for laboratories to understand the clinical implications of the results of the measurements they report and to be aware of those where UM could affect clinical interpretations and patient management.

Equation [8]:

$$UM_C = \pm\sqrt{U^2_{measurementprocedure} + U^2_{\text{calibrator}} + U^2_{Bias(\text{external control})} \cdots + U^2_{\text{other factors}}}.$$

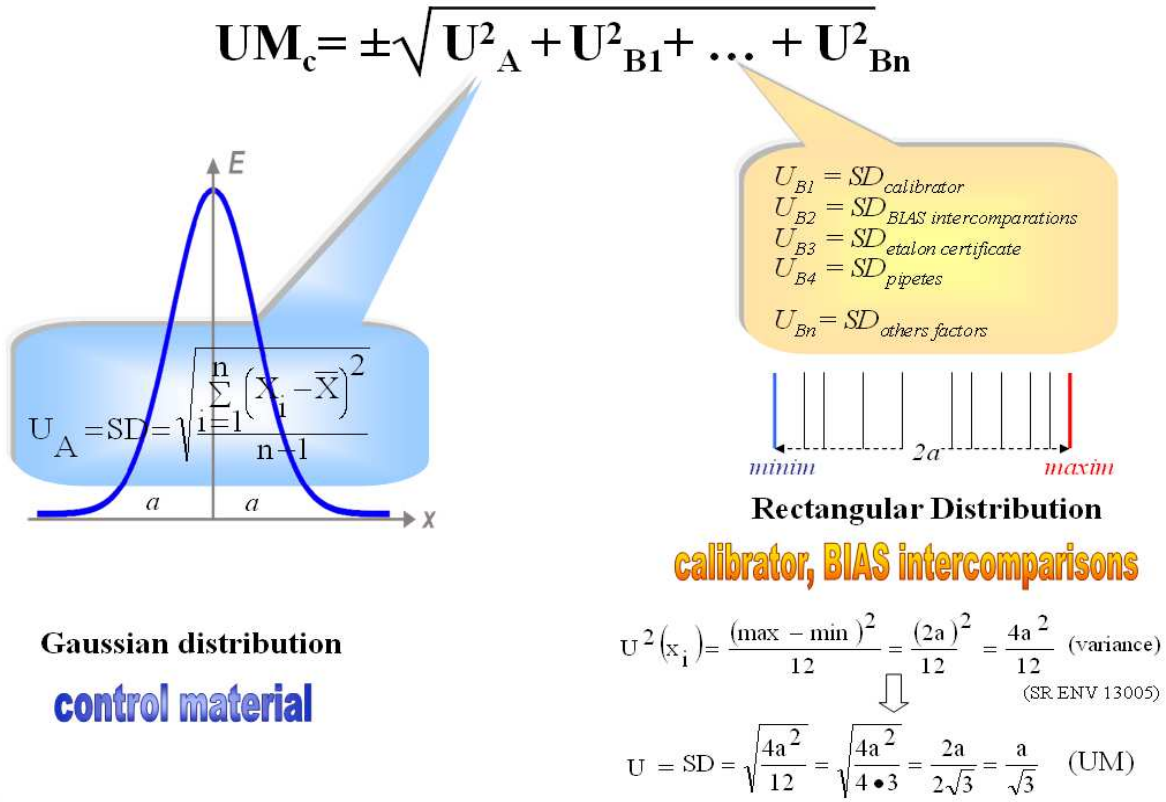


Figure 6. Combined standard uncertainty comprises type A standard uncertainty (U_A, precision of measurements) and type B standard uncertainty (U_B, accuracy of measurements).

U_A is statistically calculated from values of independent measurements with Gaussian distribution. Regarding type B uncertainty, there are many kinds of U_B according to literature specifications: $U_{B1}, U_{B2}, \ldots\ldots, U_{Bn}$. When a medical laboratory chooses to estimate its accuracy of measurements from Bias intercomparisons, the standard deviation of Bias (Bias values from many participations to external control have rectangular distribution) has to be calculated following the formula $SD_{Bias} = \frac{a}{\sqrt{3}}$, where a = Bias (adapted after SR ENV 13005:2005).

Uncertainty reported with the result is expanded uncertainty and is then conveniently expressed as:

$Y \pm U$ [10],

where:

Y = measured result,

U = expanded uncertainty.

The laboratory should offer detailed data to users (physician, patient) about the pattern estimation used for UM.

Each laboratory has the responsibility to identify all quantifiable error sources, which can be converted in standard deviations used for estimating type A and type B standard uncertainty.

Type A standard uncertainty is assessed from internal quality control data (statistical calculation on at least 30 values of control serum from at least 30 different consecutive days). Type A standard uncertainty illustrates the precision of laboratory measurements.

When the laboratory uses traceable calibrators to certified reference materials (CRM) with attributed UM, this can be treated as type B standard uncertainty. Type B standard uncertainty shows the accuracy of measurements.

Table 1. Estimation of UM when the medical laboratory has data about $UM_{calibrator}$

<table>
<tr><td>Quantity</td><td>CREATININE</td></tr>
<tr><td>Measurement</td><td>Creatinine concentration in human serum</td></tr>
<tr><td>Units</td><td>mg/dL, μmol/L</td></tr>
<tr><td>Reference intervals</td><td>Men 0,6 – 1,1 mg/dl
53 – 97 μmol/L
Women 0,5 – 0,9 mg/dl
44 – 80 μmol/L</td></tr>
<tr><td>Test principle</td><td>Creatinine in alkaline solution reacts with picrate to form a colored complex. The rate of formation of the complex is measured.</td></tr>
<tr><td>Calibrator traceability</td><td>Is traceable to the NIST reference material 909b level 2 and by the reference method IC-GC/MS</td></tr>
<tr><td>Precision / Imprecision (Type A uncertainty)</td><td>Internal quality control data on multiparameter control serum for: 1.01.2009 – 31.03.2009
<table>
<tr><td>Number of measurements</td><td>Quality control MEAN</td><td>SD</td><td>CV (%)</td></tr>
<tr><td>72</td><td>3.55 mg/dl</td><td>± 0.17 mg/dl</td><td>4.84 %</td></tr>
</table></td></tr>
<tr><td>Uncertainty of calibrator (Type B uncertainty)</td><td>Calibrator traceability
<table>
<tr><td>Calibrator</td><td>Target value</td><td>Total Uncertainty ($U_{expanded}$)</td><td>Per cent Uncertainty</td></tr>
<tr><td>Creatinine</td><td>3,6 mg/dl</td><td>0,26 mg/dl</td><td>7,1%</td></tr>
</table>
Uncertainty is calculated as the half of the 95% confidence interval of the calibrator assigned value. The true value should fall in the range (assigned value±uncertainty) with 95% probability.</td></tr>
<tr><td>UMcombined (UMc)</td><td>$\mathbf{UM_c = \pm\sqrt{U_A^2 + U_B^2}}$ The medical laboratory is responsable for taking account other types UB from apriori data beside $UM_{calibrator}$.</td></tr>
<tr><td>UA</td><td>$U_{measurement\ procedure}$ = standard deviation of quality control results for period 1.01.2009 – 31.03.2009
UA = ± 0,17 mg/dL</td></tr>
<tr><td>UB</td><td>$UM_{combined\ (calibrator)}$ obtained from manufacturer as standard deviation. The manufacturer offers $UM_{expanded}$ (for a level of confidence of 95%, k=2), so that the laboratory should divide by 2 the $UM_{expanded}$ offered by manufacturer.
$UB = UM_{combined\ (calibrator)} = \frac{UM_{expanded}}{k} = \frac{0,26}{2} = \pm 0,13$ mg/dL</td></tr>
<tr><td>UMc</td><td>$UMc = UM_{combined}$
$UMc = \pm\sqrt{SD^2_{(precision\ from\ internal\ control)} + UM^2_{combined\ (calibrator)}} =$
$= \pm\sqrt{0,17^2 + 0,13^2} = \pm\sqrt{0,30^2} = \pm 0,30$
Note:
● the traceability of calibrators to CRM provides the accuracy of measurements;
● in this case, the usage of other types of UB is supplementary, but not mandatory because the traceability of calibrators to CRM covers the accuracy of measurements (Figure 6);
● the proportion brought by other types of UB to the UMc is lower. The medical laboratory could choose whether to take into account the Ubias (from external control) beside U calibrator for calculation of UMc.</td></tr>
<tr><td>U</td><td>$U = UM_{expanded} = UMc \cdot k = \pm 0,30 \cdot 2 = \pm 0,60$
k =2, for level of confidence of 95%</td></tr>
<tr><td>Reporting UM</td><td>Y ± U, where
Y = measured result
U = expanded uncertainty
Mean value of creatinine 3.55 ± 0,60 mg/dl</td></tr>
</table>

Unfortunately, in most medical laboratories from our country calibrators without traceable values are used. If an appropriate reference material or reference procedure is unavailable, then alternative approaches may be used, e.g. external quality assessment data or inter-laboratory comparisons (13).

In this case, accuracy of measurements is estimated by type B uncertainty calculated as standard deviation of Bias values from external control. Bias is the difference between measured result and average results of participant medical laboratories based on an intercomparisons scheme (external control). Bias values from many external control participations have a rectangular distribution (variance) given by:

$$U^2(x_i) = \frac{(\max - \min)^2}{12} = \frac{\left(2a^2\right)}{12} = \frac{4a^2}{12}$$ [11] (6).

For this reason, standard deviation of Bias is calculated by formula:

$$U = SD = \sqrt{\frac{4a^2}{12}} = \sqrt{\frac{4a^2}{4 \bullet 3}} = \frac{2a}{2\sqrt{3}} = \frac{a}{\sqrt{3}}$$ [12],

(*Figure 6*).

In this paper the authors summarize the estimation of UM in two situations: when the assigned values of traceable calibrators to CRM are or not accompanied by UM as shown in *Tables 1 and 2*.

The assigned values of traceable calibrators to CRM are accompanied by UM as shown in *Table 1*.

Manufacturers do not always provide the uncertainty of the values assigned to calibrators. In this case UM is estimated by following the model shown in *Table 2*.

Conclusions

1. Uncertainty Measurement of a measured result increases its reliability.
2. A reasonable estimation of UM includes precision (UA) and accuracy of measurements (UB):
   a. Precision is expressed as standard deviation of the values obtained in internal quality control using a large number of measurements on at least 30 days period.
   b. Measurements results in medical laboratories are used for clinical decisions, and therefore it is very important to include accuracy of measurement in $UM_{combined}$. Each medical laboratory has the responsibility to guarantee the accuracy of measurements by using reference materials (calibrators) with traceability to CRM and / or participations of the laboratory to intercomparison schemes.
3. The laboratories should request data about the traceability of calibrators from the manufacturers. Uncertainty of the value assigned to the calibrator has a major importance in assessing measurement accuracy.
4. When the medical laboratory does not have data about the calibrator values' UM, the accuracy of measurements could be estimated by standard deviation of Bias from external quality control (intercomparison schemes).
5. It is important for laboratories to understand the clinical implications of the results of the measurements they report and to be aware of those results where UM could affect clinical interpretations and patient management. The clinician needs to be aware of the UM together with the result to make a correct diagnosis. The uncertainty of the result is important, e.g. when looking at the limits of reference interval or cut off. It is necessary to estimate the UM when comparing results to allowable values, e.g. tolerance limits or allowable (legal) concentrations (doping control).
6. Where uncertainty of measurement information, if reported, could significantly affect clinical interpretations and patient management (e.g. tumor marker monitoring), such information should be readily available on request and comprehensive about the laboratory's method of assessment.

Table 2. Estimation of UM when the medical laboratory does not have data about $UM_{calibrator}$

<table>
<tr><td>Quantity</td><td>Tyroid stimulating hormone (TSH)</td></tr>
<tr><td>Measurement</td><td>TSH concentration in human serum.</td></tr>
<tr><td>Units</td><td>µUI/mL</td></tr>
<tr><td>Reference intervals</td><td>0,4 – 6,0 µUI/mL</td></tr>
<tr><td>Test principle</td><td>The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique mouse monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule.</td></tr>
<tr><td>Calibrator traceability</td><td>6 calibrators (included in kit): 0; 0,5; 2; 5; 10; 25 µUI/ml
is not traceable to CRM</td></tr>
<tr><td>Precision / Imprecision (Type A uncertainty)</td><td>internal QC data on multiparameter control serum for: 1.01.2009 – 31.03.2009
<table>
<tr><td>Number of measurements</td><td>QC Mean</td><td>SD</td><td>CV (%)</td></tr>
<tr><td>48</td><td>4.03 µUI/ml</td><td>± 0.32 µUI/ml</td><td>7.94%</td></tr>
</table></td></tr>
<tr><td>Type B uncertainty</td><td>Standard deviation of Bias from external quality control (intercomparisons schemes)
Bias = measured result – intercomparison average
The Bias values from the same period for $U_{measurement\ procedure}$ have a rectangular distribution, so
$SD_{Bias} = \frac{a}{\sqrt{3}}$ (Figure 6)
BIAS1 (Cycle 6/Sample 8) = 2.98 – 3.02 = - 0,04
BIAS2 (Cycle 6/ Sample 10) = 3.64 – 3.81 = - 0,17
BIAS3 (Cycle 6/ Sample 11) = 4,26 – 4,20 = +0,06
$SD_{Bias} = \frac{a}{\sqrt{3}} = \frac{BIAS\ \max.}{\sqrt{3}} = \frac{-0,17}{\sqrt{3}} = 0,09\ \mu UI/ml$</td></tr>
<tr><td>UMc</td><td>$UMc = UM_{combined}$
$UM_c = \pm\sqrt{U_A^2 + U_B^2}$
$UMc = \pm\sqrt{SD^2_{(precision\ from\ internal\ control)} + SD^2_{Bias(external\ control)}} =$
$= \pm\sqrt{0.32^2 + 0,09^2} = \pm 0,33\ \mu UI/ml$
Note: The medical laboratory is responsible for taking into account other UB types from apriori data beside UM_{Bias} (from external control)</td></tr>
<tr><td>U</td><td>$U = UM_{expanded} = UMc \cdot k = \pm 0,33 \cdot 2 = \pm 0,66$ µUI/ml
k =2, for level of confidence of 95%</td></tr>
<tr><td>Reporting UM</td><td>Y ± U, where
Y = measured result
U = expanded uncertainty
Mean value of TSH 4.03 ± 0,66 µUI/ml</td></tr>
</table>

7. Assessing and reporting measurement uncertainty will help reduce the differences between laboratories' results, which translates into prompt clinical decision, low costs, higher efficiency and highest confidence of clinicians and patients in testing results.

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Abbreviations list

CRM	certified reference materials
CV	coefficient of variation
EA	European Accreditation
GUM	Guide to the expression of uncertainty in measurement
OD	optical density
SD	standard deviation
UM	uncertainty of measurement
UMc	combined standard uncertainty
$UM_{expanded,}$ U	expanded standard uncertainty

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