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Estimation Methods for Various Cases of Uncertainty of Measurement in Clinical Chemistry

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Abstract

Regarding the expression of the reliability of measurement results, an international consensus is emerging that analytical values are expressed in combination with the uncertainty of their measurement. This paper proposes basic procedures for the estimation of uncertainty in analytical methods used for laboratory testing. There are two situations where uncertainty estimation is necessary: (1) estimation of the uncertainty of routine test values and (2) estimation of the uncertainty of assigned values of calibrators and quality assurance (QA) samples. For each of these situations, methods of uncertainty estimation are discussed in four cases: the cases where the analytical procedures are calibrated at every batch of assay, where the calibration takes place only at the first batch of assay, where multipoint linear calibration is performed, and where calibration is performed using a calibration curve.

Key words: uncertainty of measurement, estimation method, routine test value, assigned value of calibrator and quality assurance sample, traceability, metrology, reliability, measurand, component of uncertatinty, reference material, linear calibration, nonlinear calibration, repeatability, reproducibility, measurement condition, standard deviation, standard uncertatinty, expanded uncertatinty, coverage factor, analysis of variance, error of measurement, measurement procedure.

Abbreviations: ISO, International Organization for Standardization; GUM, guide to the expression of uncertainty in measurement; ANOVA, analysis of variance; SD, standard deviation; QA, quality assurance.

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1. Introduction

1.1 Background

"Guide to the Expression of Uncertainty in Measurement: GUM"[1], an international document edited at the initiative of the International Organization for Standardization (ISO), has been published to provide guidance concerning the expression of the reliability of measurement results. In the field of chemical analysis, an international consensus based on this concept is emerging that

analytical values are expressed in combination with the uncertainty of their measurement to indicate their reliability.

On the basis of the international document, the Committee on Quality Management of the Japan Society of Clinical Chemistry released a guideline titled "Evaluation Protocols of Estimating Uncertainty for Calibrators and Control Materials Used for Quality Assurance (QA)"[2]. The publication provides general considerations on uncertainty estimation in various cases of calibration in absorptiometry: (1) calibration using standard solutions, (2) calibration using a reference material, and (3) calibration using reference materials with different concentrations.

1.2 Purpose

In this paper, procedures for uncertainty estimation are described with mention to specific cases of their applications to the estimation of uncertainty in practical settings. However, the theoretical background for uncertainty estimation and particular considerations for its implementation are not discussed herein.

1) Basic experiments and analysis of variance for uncertainty estimation
2) Uncertainty of routine test values
2.1) Estimating the uncertainty of routine test values
2.2) Estimating the uncertainty of routine test values using internal quality control data
2.3) Estimating the uncertainty of routine test values with calibration components calculated separately.
3) Uncertainty of assigned values of calibrators and QA samples
3.1) Estimating the uncertainty of assigned values of calibrators and QA samples
3.2) Estimating the uncertainty of assigned values of calibrators and QA samples with the calibration components calculated separately
4) Estimating the uncertainty in measurement with multipoint linear calibration
5) Estimating the uncertainty in measurement using a calibration curve

2. General Procedures for Uncertainty Estimation[1-3]

(1) Establish measurement and calibration procedures and describe the principle of measurement, method of measurement, measuring instrumentation, measurement procedures and others for short. In cases where the mean value of multiple measured values is obtained, also describe the data processing procedures clearly. Here, a measured value refers to the best estimate (y) of a measurand.

(2) Formulate the measurement/calibration method in the form of a theoretical or empirical formula. If it cannot be expressed in the form of a numerical formula, enumerate the factors of uncertainty, estimate the uncertainty for each factor, and combine all the uncertainty components.

(3) For parameters requiring a correction of measured values, such as temperature correction, estimate the uncertainty of corrected data.

(4) Enumerate and classify the components of uncertainty and estimate "standard uncertainty" by expressing the magnitude of uncertainty for each component as standard deviation (or equivalent). The components of uncertainty are classified into two types according to method of their estimation: type A comprises the components of uncertainty estimated using statistical methods, and type B comprises the components of uncertainty estimated using prior art information, experience, certificate of performance and the like.

(5) Calculate the square root of the sum of the squares of the standard uncertainty for component [$u_C = (\Sigma u_i^2)^{1/2}$] to obtain "combined standard uncertainty."

(6) Multiply the combined standard uncertainty by the coverage factor (k) to obtain "expanded uncertainty [$U = k\ u_C$]." Generally, assume $k = 2$ and a confidence level of $p = 95\%$, then obtain expanded uncertainty [$U = 2\ u_C$].

Provided that the standard uncertainty is obtained as a relative value, the expanded uncertainty is expressed as [$U = 2 \times y \times$ relative value of combined standard uncertainty].

(7) Express the uncertainty as "$y \pm U$, assuming that the coverage factor is $k = 2$, and that the combined standard uncertainty is u_C."

Regarding the number of places in the numerical expression of uncertainty, the number of places used in the ordinary expression of the measured value is acceptable; however, it is desirable that one more place be added, in view of the possible use of the estimated value of uncertainty by others.

(8) Record the data evaluated for uncertainty in an open document.

3. Measurement/Calibration Procedures for Measurands

The methods proposed in this paper apply to estimate the uncertainty of measured values of the target component (or enzyme activity or the like) concentrations obtained by absorptiometry. The absorbances (or absorbance changes, written as *A*s and *A*b, respectively) of a reference material having a value of *C*s and a blank sample (blank determination) are determined. A calibration line (working line?) is generated using the measured values, and the absorbance (*A*x) of an unknown sample is substituted for the following equation to obtain the value of the unknown sample (*C*x).

$$Cx = Cs \times [(Ax - Ab)/(As - Ab)]$$

Cases of multipoint linear calibration and cases of calibration curves should be handled separately.

The reference material used should be an actual sample, the property of which is similar to the patient specimen to be assayed.

4. Classification of Components of Uncertainty

Although there are a variety of factors involved in uncertainty in chemical measurement, including sample weighing and volumetry, measuring operations, reference material, and interfering substances[3], they can roughly be divided into three types in case of absorptiometry with linear calibration as shown below.

(1) Uncertainty of reference material: Uncertainty of certified values. If stability before and after unpacking is specified, combined standard uncertainty including those data shall be estimated.

(2) Uncertainty of sample and sample preparation: Errors, heterogeneity (between-vial differences) and the like associated with thawing, dissolution, and dilution.

(3) Uncertainty associated with measuring operations: Calibration dispersion, within-day and between-day dispersion, within-laboratory and between-laboratory dispersion, and the like, including factors due to reagent preparation and instrument variation. These factors usually occur as type A components and can be estimated by repeating a measurement experiment. It should be noted that uncertainty estimation procedures differ between cases where the analytical procedures are calibrated at the time of every repeat, and cases where the calibration takes place at given intervals.

In addition, the analytical procedures used here shall have been verified as being free from the matrix effect of the sample and the influence of interfering substances.

5. Estimation of Components of Uncertainty

5.1 Uncertainty of reference material (standard uncertainty = u_S, or its relative value)

The expanded uncertainty (Us) of the labeled value of reference material (Cs) is generally expressed by the following equation, and is a type B factor, which is usually specified in the Written Certificate.

Uncertainty of labeled value: $C\text{s} \pm U\text{s}$

Provided that Us is shown by expanded uncertainty using coverage factor k, standard uncertainty (u_S) is obtained as $u_S = U\text{s}/k$.

If Us is shown as $\pm U$s (%, tolerance) indicating the maximum and minimum values, it is deemed a rectangular distribution (uniform distribution) and standard uncertainty is estimated as $u_S = U\text{s}/\sqrt{3}$.

5.2 Uncertainty of sample and sample preparation (standard uncertainty = u_B, or its relative value)

Sample heterogeneity due to sample preparation operations such as thawing and dissolution and between-vial differences can be estimated in experiments performed to quantify the uncertainty associated with measuring operations described below.

5.3 Uncertainty associated with measuring operations (standard uncertainty = u_M, or its relative value)

Measurement conditions are roughly divided into within-laboratory and between-laboratory measuring conditions.

Under within-laboratory measuring conditions, the uncertainty due to between-day variation (u_A) and the uncertainty due to within-day variation (u_E) are estimated from repeatedly measured values of the test sample within the same laboratory. Under between-laboratory measuring conditions, the uncertainty due to between-laboratory variation (u_A) and the uncertainty due to within-laboratory variation (u_E) are estimated from simultaneously measured values of the test sample obtained at more than one laboratory. In both cases, the individual components are composed to obtain standard uncertainty due to measurement conditions (u_M).

$$u_M = (u_A^2 + u_E^2)^{1/2}$$

These uncertainties due to measurement conditions include sample heterogeneity, reagent preparation errors, and errors due to analyzer-related variation. In addition, when a calibration line is generated at the time of every measurement under within-laboratory measuring conditions, between-day variation includes errors associated with calibration, whereas when a calibration line is generated at given intervals with no calibration performed during that period, it is necessary to separately quantify errors associated with calibration and combine them with between-day variation during the calibration intervals.

The magnitude of between-day/within-day variation can also be estimated from the internal quality control data obtained daily at the laboratory, as described below.

6. Basic Experiments and Analysis of Variance for Uncertainty Estimation

6.1 Experiments for estimation of within-laboratory uncertainty

Uncertainty associated with measuring operations within the same laboratory is estimated by applying the nested analysis of variance method[4] through experimental data with between-day/within-day variation and sample vial as relevant factors.

Specifically, a calibration line is generated at the time of every experiment during p days (times) of the experimental period, and each of q vials of test sample is repeatedly measured n times to obtain $p \times q \times n$ measured values (Figure 1). The number of measurement days (times) is desirably not less than 15, and the minimum number of vials or repeats for each measurement day is 2 [5].

The thus-obtained measured values are examined for outliers. If outlier(s) is found, its cause is identified and the value is removed. If a problematic finding is obtained in the measurement, a new measurement is performed. After these investigations, two-stage nested analysis of variance is applied to estimate individual variation components, i.e., between-vial variation, between-day variation, and within-day variation.

6.2 Experiments for estimation of between-laboratory uncertainty

Between-laboratory uncertainty is estimated by following the above-described experimental procedures, but performing experiments at p laboratories, in place of repeating experiments for p days, repeatedly measuring each of q vials of sample n times at each laboratory, and analyzing the thus-obtained data using the procedures shown below.

6.3 Two-stage nested analysis of variance

Measured values are expressed using the following equation model:

$$X_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk}$$

where $i = 1, \ldots, p$; $j = 1, \ldots, q$; $k = 1, \ldots, n$

Here, X_{ijk} is the k-th value for vial j measured on measurement day i (or at laboratory i, hereinafter factors for between-laboratory experiments are given in parentheses).

The symbol μ represents overall mean, α_i represents between-day (-laboratory) variation for measurement day (laboratory) i, β_{ij} represents vial j variation for measurement i, and ε_{ijk} represents within-day (-laboratory) variation. Here, variance of between-day (-laboratory) variation is written as σ_A^2, variance of between-vial variation as σ_B^2, and variance of within-day (-laboratory) variation as σ_E^2.

The calculation procedures are described below. First, totals T_{ij}, T_i, and T, and means XB_{ij}, XB_i, and XB for individual vials, individual days (laboratories), and all vials or days (laboratories), respectively, are obtained.

$$XB_{ij} = (\Sigma_k X_{ijk})/n = (T_{ij})/n$$

$$XB_i = (\Sigma_j T_{ij})/(qn) = (T_i)/(qn) = (\Sigma_j XB_{ij})/q$$

$$XB = (\Sigma_i T_i)/(pqn) = T/(pqn) = (\Sigma_i XB_i)/p$$

Next, total variation S_T, between-day (-laboratory) variation S_A, between-vial variation S_B, and within-day (-laboratory) variation S_E are calculated using the following corrected function CF, and their respective degrees of freedom f_T, f_A, f_B, f_E are calculated.

$$CF = (\Sigma\Sigma\Sigma X_{ijk})^2/(pqn) = T^2/(pqn)$$

$$S_T = \Sigma\Sigma\Sigma(X_{ijk} - XB)^2 = \Sigma\Sigma\Sigma X_{ijk}^2 - CF$$

$$f_T = pqn - 1$$

$$S_A = qn\Sigma(XB_i - XB)^2 = (\Sigma T_i^2)/(qn) - CF$$

$$f_A = p - 1$$

$$S_B = n\Sigma\Sigma(XB_{ij} - XB_i)^2 = (\Sigma\Sigma T_{ij}^2)/n - CF - S_A$$

$$f_B = p(q-1)$$

$$S_E = \Sigma\Sigma\Sigma(X_{ijk} - XB_{ij})^2 = S_T - S_A - S_B$$

$$f_E = pq(n - 1)$$

Unbiased variance for each factor is calculated.

$$V_A = S_A / f_A, \quad V_B = S_B / f_B, \quad V_E = S_E / f_E$$

The above results are summarized in the table of analysis of variance (Table 1).

Overall mean μ and variance for each variation component can be estimated as shown below. The symbol ^ below indicates an estimate.

$$\mu\hat{} = XB, \quad \sigma_A^{2}\hat{} = (V_A - V_B)/qn$$

$$\sigma_B^{2}\hat{} = (V_B - V_E)/n, \quad \sigma_E^{2}\hat{} = V_E$$

Here, $\sigma_A\hat{}$ represents an estimate of between-day (-laboratory) uncertainty u_A, $\sigma_B\hat{}$ represents an estimate of between-vial uncertainty u_B, and $\sigma_E\hat{}$ represents an estimate of within-day (-laboratory) uncertainty u_E. If the value of $\sigma_A^{2}\hat{}$ or $\sigma_B^{2}\hat{}$ is negative, it shall be handled as zero.

Between-day (-laboratory) variation is judged to be significant, if the following statistic:

$$F = V_A / V_B$$

has a value exceeding the *F*-distribution value of the degrees of freedom (p-1) and $p(q$-1). If between-day (-laboratory) variation is insignificant, the relevant factor is assumed to have no effect and handled as repeated data.

The significance of between-vial variation is determined by comparing:

$$F = V_B / V_E$$

with the *F*-distribution value of the degrees of freedom $p(q$-1) and $pq(n$-1). If between-vial variation is insignificant, the relevant factor is assumed to have no effect and handled as repeated data.

7. Uncertainty of routine test values

7.1 Estimating the uncertainty of routine test values

Experiments and data analysis are performed in accordance with "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation" with a control material such as pooled serum as the test sample. Regarding the values for the control material as the test sample, it is desirable that three (low, intermediate and high) concentrations of the control material be provided to represent patient specimens, which can have a broad range of values, and that the same experiment be performed for each concentration to obtain estimates of the uncertainties for the different concentrations in advance. Using the thus-obtained estimates of uncertainties (u_A, u_B, u_E), the uncertainty of routine test values is calculated.

If an ordinary commercially available kit is used for measurement, the reference material used is a calibration reference material attached to the kit. Not only the labeled value but also the value of its standard uncertainty (u_S) shall be confirmed in advance.

A measured value of a patient specimen in routine testing is usually obtained from a single measurement, and the uncertainty of the single measurement (u_C) is quantified by combining the uncertainty of calibration reference material (u_S), the uncertainty of sample homogeneity (u_B), and the uncertainty due to measurement conditions including calibration ($u_M = (u_A^2 + u_E^2)^{1/2}$), as shown below.

$$u_C = (u_S^2 + u_B^2 + u_M^2)^{1/2}$$

Hence, the uncertainty of a measured value obtained from a given measurement is derived from the sum of the squares of the variances of the individual components of uncertainty, i.e., reference material, sample homogeneity, and measurement conditions.

7.2 Estimating the uncertainty of routine test values using internal quality control data

In estimating the uncertainty of routine test values, cumulative internal quality control data collected everyday at the laboratory can be used. It is necessary, however, that the measurement should be stable with the batch of the control material remaining unchanged during the period of compiling measured values of the control material.

One-way layout analysis of variance is applied to measured values of the control material obtained at constant frequency (≥ 2 times) daily over a period of two to three months, so as to obtain the standard uncertainties of both between-day and within-day variation components in the

measurement (u_A, u_E). These uncertainties due to measurement conditions include those due to sample heterogeneity and calibration.

Adding the uncertainty of the calibration reference material (u_S) to the thus-quantified uncertainties due to measurement conditions, the uncertainty of routine test values is calculated as shown below.

$$u_C = (u_S^2 + u_A^2 + u_E^2)^{1/2}$$

As in the case of "7-1. Estimating the uncertainty of routine test values," it is desirable that the magnitude of uncertainty be determined for each of three (low, intermediate, high) concentrations of the control material.

7.3 Estimating the uncertainty of routine test values with the calibration components calculated separately

The uncertainty of routine test values is estimated in three components: uncertainty of reference material (u_S), uncertainty of sample homogeneity and measurement conditions (u_B, u_M'), and uncertainty of calibration (u_{CAL})[6]. The uncertainty of reference material is expressed by the value specified in the Written Certificate. The uncertainty due to measurement conditions is estimated using procedures in accordance with "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation," except that calibration during the experimental period is performed only once at the first time of measurement. The uncertainty of calibration is estimated from the data used to generate the calibration line at the first time of measurement, using the *SN* ratio[7] in accordance with the procedures shown below.

(1) Estimating the uncertainty associated with calibration

Write the reading (absorbance, analyzer output value, etc.) obtained by analyzing m units of reference material having values of x_i (i = 1, 2, ... , m) (including blank determinations) in n repeats as y_{ij} (i = 1, ... , m; j = 1, ..., n). Here, cases where a calibration line ($y_{ij} = \alpha + \beta x_i + \varepsilon_{ij}$) is assumable between x and y are handled. Here, α represents the zero point bias, β represents the gradient of the calibration line (also referred to as sensitivity coefficient), and ε represents the error of the calibration line; it is assumed that variance σ^2 is constant, and that the error includes deviations from the linearity and the like.

The error variance of measured values obtained with appropriate calibration using a reference material is calculated using the *SN* ratio as described below. Specifically, the total variation of data S_T is resolved into the variation from measurand S_B, the magnitude of generalized mean effect S_m, and error variation S_E ($S_T = S_B + S_m + S_E$). The individual variations, effective divisor r, and error variance V_E are calculated as shown below.

$$S_T = \Sigma\Sigma\, y_{ij}^2, \quad S_m = (\Sigma\Sigma\, y_{ij})^2/(mn)$$

$$r = n\Sigma(x_i - XB)^2, \text{ where } XB = (1/m)\Sigma\, x_i$$

$$S_B = \{\Sigma(x_i - XB)\, y_i\}^2 / r, \text{ where } y_i = \Sigma\, y_{ij}$$

$$S_E = S_T - S_m - S_B$$

$$V_E = S_E/(mn - 2)$$

From these values, the *SN* ratio (η) can be calculated using the equation shown below.

$$\eta = \beta^2/\sigma^2 = (1/r) \cdot (S_B - V_E) / V_E$$

The *SN* ratio is the quotient obtained by dividing the magnitude of the sensitivity of the calibration line by the error variation. The reciprocal of the *SN* ratio is an estimate of the error variance of measured values with appropriate calibration. Using the *SN* ratio, the uncertainty associated with calibration is calculated as shown below.

$$u_{CAL} = 1/\sqrt{(\eta)}$$

(2) Combining the components of uncertainty

Using estimates of the uncertainty associated with calibration obtained above, the uncertainty of reference material, and the uncertainty of measurement obtained in accordance with "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation," the combined standard uncertainty of routine test values is calculated as shown below.

$$u_C = (u_S^2 + u_{CAL}^2 + u_B^2 + u_M'^2)^{1/2}$$

8. Uncertainty of assigned values of calibrators and QA samples

8.1 Estimating the uncertainty of assigned values of calibrators and QA samples

With a calibrator or a QA sample as the test sample, experiments and data analysis are performed in accordance with "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation."

From the results, the labeled value of the calibrator or the QA sample is obtained as μ^.

Using estimates of uncertainties for between-day (-laboratory) variation, between-vial variation, and within-day (-laboratory) variation obtained from the experiments (u_A, u_B, u_E), and the value of the uncertainty of the reference material (u_S), the combined standard uncertainty of the labeled value (u_C') is calculated as shown below.

$$u_C' = (u_S^2 + u_A^2/p + u_B^2/pq + u_E^2/pqn)^{1/2}$$

It seems that the value of the calibrator or the QA sample as the test sample and the value of the reference material used for analytical procedure calibration often differ from each other. In such cases, the above calculation is performed using the relative value of the standard uncertainty, and the thus-obtained value is multiplied by the labeled value to obtain combined standard uncertainty.

8.2 Estimating the uncertainty of assigned values of calibrators and QA samples with the calibration components calculated separately

With a calibrator or a QA sample as the test sample, the labeled value and its uncertainty are determined. Here, the uncertainty is estimated in three components: the uncertainty of reference material (u_S), the uncertainty of sample heterogeneity, between-day variation, and within-day variation (u_B, u_A', u_E'), and the uncertainty of calibration (u_{CAL}). The uncertainty due to measurement conditions is estimated in the same manner as "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation," except that calibration during the experimental period is performed only once at the first time of measurement. The uncertainty of calibration is calculated in accordance with "7.3 Estimating the uncertainty of routine test values with the component due to

calibration calculated separately" using the data used to generate the calibration line at the first time of measurement.

Using the thus-obtained estimates of the uncertainties for the individual components, the uncertainty of the labeled value of the calibrator or the QA sample is calculated as shown below.

$$u_C' = (u_S^2 + u_{CAL}^2 + u_A'^2/p + u_B^2/pq + u_E'^2/pqn)^{1/2}$$

In this case, the analytical procedures are calibrated only once at the first time of measurement of the calibrator or the QA sample; it is necessary to technically assure the absence of a significant bias of trueness in the second measurement and thereafter.

9. Estimation of Uncertainty in Measurement Using Multipoint Linear Calibration

9.1 Uncertainty of routine test values

Even in the case of measurements requiring multipoint linear calibration with three or more ($m \geq 3$) different concentrations of reference material, the uncertainty of routine test values can be quantified using basically the same procedures as "7.1 Estimating the uncertainty of routine test values," except that the uncertainty of the reference material is calculated as a combined standard uncertainty using the mean value obtained by averaging the relative values of m kinds of standard uncertainties (or maximum value).

Alternatively, the uncertainty can be estimated in three components in accordance with "7.3 Estimating the uncertainty of routine test values with the component due to calibration calculated separately": the uncertainty of reference material (u_S), the uncertainty of sample homogeneity and measurement conditions (u_B, u_M'), and the uncertainty due to calibration (u_{CAL}). In the case of multipoint linear calibration, the same data analysis procedures are applicable. For the uncertainty of routine test values, combined standard uncertainty is calculated using estimates of the uncertainty due to calibration, the uncertainty of reference material, and the uncertainty due to measurement conditions obtained in accordance with "6. Basic Experiments and Analysis of Variance for Uncertainty Estimation."

9.2 Uncertainty of calibrator or QA sample

The uncertainty of the labeled value of a calibrator or a QA sample in measurement with multipoint linear calibration can also be determined using basically the same procedures as "8.1 Estimating the uncertainty of assigned values of calibrators and QA samples," except that the uncertainty of reference material is calculated as a combined standard uncertainty using the mean value obtained by averaging the relative values of m kinds of standard uncertainties (or maximum value).

Alternatively, this uncertainty can be estimated in three components in accordance with "8.2 Estimating the uncertainty of assigned values of calibrators and QA samples with the component due to calibration calculated separately": the uncertainty of reference material (u_S), the uncertainty of sample homogeneity and measurement conditions (u_B, u_M'), and the uncertainty due to calibration (u_{CAL}).

10. Estimation of Uncertainty in Measurement Using a Calibration Curve

In immunochemical analyses and the like, the working(calibration?) line generated can be nonlinear or a curve. In such cases, “7.1 Estimating the uncertainty of routine test values” or “8.1 Estimating the uncertainty of assigned values of calibrators and QA samples” is applicable. However, the uncertainty of reference material is calculated as a combined standard uncertainty using the mean value obtained by averaging the relative values of m kinds of standard uncertainties (or maximum value).

Provided that the reference material used for calibration is analyzed as the test sample, and the measured value is taken as the reading for the analytical procedures, the method described in “7.3 Estimating the uncertainty of routine test values with the calibration components calculated separately” or “8.2 Estimating the uncertainty of assigned values of calibrators and QA samples with the calibration components calculated separately” is applicable.

11. Conclusion

Some methods of estimating the uncertainty of routine test values and the uncertainty of assigned values of calibrators and QA samples have been described with classification according to calibration procedures for analytical procedures. Although the basic procedures used to estimate uncertainty are quite simple, a key point resides in how to appropriately characterize and evaluate the various components of uncertainty involved in the measurement process, including calibration procedures.

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Figure 1. Two-Stage Nested Experimental Data

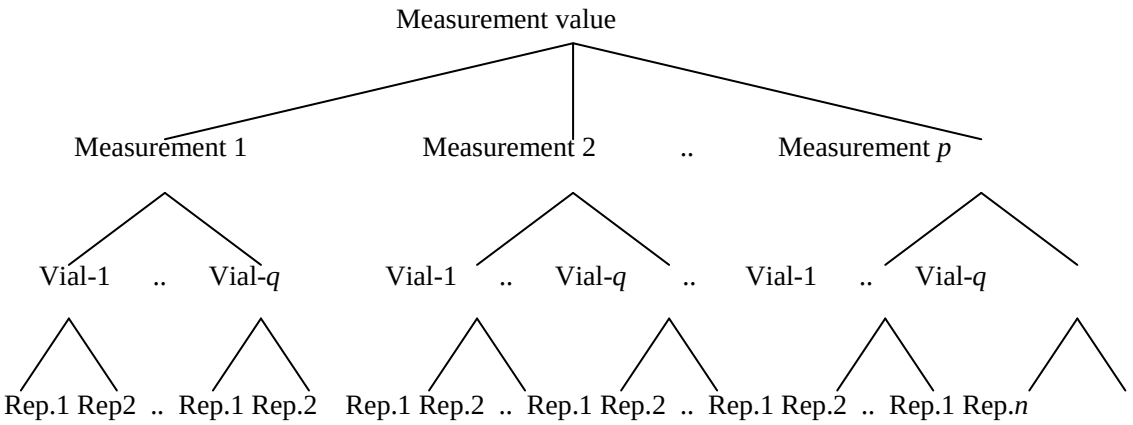

Table 1. Table of Analysis of Variance

Factor	Variation	Degree of freedom	Unbiased variance	Expectancy of unbiased variance
Between-day (-laboratory) difference	S_A	p-1	V_A	$\sigma_E^2 + n\sigma_B^2 + qn\sigma_A^2$
Between-vial difference	S_B	$p(q$-1)	V_B	$\sigma_E^2 + n\sigma_B^2$
Within-day (-laboratory) error	S_E	$pq(n$-1)	V_E	σ_E^2