Uncertainty of Measurement in Clinical Laboratory Sciences

To the Editor:

Random and systematic errors can act together to produce an error of measurement (total error) and generate a doubt (uncertainty) about the true value of the measured quantity. The international metrological organizations, keeping in mind these facts, have developed the concept of uncertainty of measurement. This concept has become an important issue in general metrology, and by extension, its importance is increasing in clinical laboratory sciences. It is thus important to clarify the concept and to identify the practical difficulties in the use of uncertainty of patients’ results.

Uncertainty of measurement (hereafter referred to as uncertainty) is a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand (i.e., the measured quantity) \( (1) \); in other words, uncertainty is numerical information that complements a result of measurement, indicating the magnitude of the doubt about this result. Uncertainty is described by means of one of the following three parameters \( (2) \):

- “Standard uncertainty” \( (u) \) is the standard deviation that denotes the uncertainty of the result of a single measurement.
- “Combined standard uncertainty” \( (u_c) \) is the standard deviation that denotes the uncertainty of the result obtained from other results of measurement. It is obtained by combining the standard uncertainties of all individual measurements according to the law of propagation of uncertainty.
- “Expanded uncertainty” \( (U) \) is the statistic defining the interval within which the value of the measured is believed to lie with a particular level of confidence. It is obtained by multiplying the combined standard uncertainty by a coverage factor, \( k \), the choice of which is based on the level of confidence \( (1 - \alpha) \) desired. If \( k = 2 \), then \( 1 - \alpha \approx 0.95 \); if \( k = 2.6 \), then \( 1 - \alpha \approx 0.99 \).

The international scientific and standardization bodies recommend that the uncertainty of patients’ results obtained in clinical laboratories should be known \( (3-5) \); the rationale for this recommendation is that full interpretation of the value of a quantity obtained by measurement also requires evaluation of the doubt attached to its value. The common opinion of these bodies is that clinical laboratories should supply information about the uncertainty of their results of measurement when applicable; ideally, this information should be attached to the patients’ results as shown in this example:

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S – \text{Alanine aminotransferase; cat.c.} = (1.15 \pm 0.23) \text{ \( \mu \)} \text{kat/L, where 1.15 \( \mu \)} \text{kat/L is the result given by the system of measurement, and 0.23 \( \mu \)} \text{kat/L is the expanded uncertainty multiplied by 2 as coverage factor. (According to IFCC and IUPAC, S is serum, and cat.c. is the catalytic concentration.)}
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Institutional guidelines for estimating uncertainty of measurement, containing examples in fields of application other than clinical laboratory sciences, have been published \( (2, 6-8) \). An excellent review of uncertainty (and traceability) in clinical chemistry was published recently \( (9) \).

Depending on the field of application, uncertainty is attributable to different sets of elements. Each element of uncertainty, expressed as a standard deviation, may be estimated from the probability distribution of values with repeated measurements, termed “type A standard uncertainty”, or estimated by use of an assumed probability distribution based on experience or other available information, termed “type B standard uncertainty.”

In general, in clinical laboratory sciences the most relevant elements that can contribute to uncertainty for a given system of measurement are:

- Incomplete definition of the particular quantity under measurement,
- Unrepresentative sampling,
- Withdrawal conditions,
- Effects of additives,
- Centrifugation conditions,
- Storage conditions,
- Day-to-day (or between-run) imprecision,
- Systematic error,
- Lack of specificity,
- Values assigned to calibrators

Estimation of the combined uncertainty, expressed as a variance, is the sum of the values, all expressed as variances, corresponding to several of the above elements. Perhaps variances corresponding to these elements can be easily estimated in some clinical laboratories, but for others their evaluation is certainly not easy, as may be derived from the following points:

(a) Manufacturers do not give the uncertainty of the values assigned to calibrators.
(b) In the majority of measurement procedures used in clinical laboratories, the metrological standard deviation varies with the value of the measurand; this phenomenon, called “heteroscedasticity” (the opposite is called homoscedasticity), should be always taken into account when estimating uncertainty.
(c) Premetrological variation should not be considered negligible even when the premetrological process seems to be well standardized \( (10, 11) \).

Bearing in mind these points, the following questions arise:

- When will manufacturers supply the uncertainties of the values assigned to calibrators?
- How many clinical laboratories know—or really can know—the mathematical or graphical relationship between metrological standard deviation and concentration for each measurement procedure?
- How many clinical laboratories know—or really can know—the standard deviation of their premetrological variation for each quantity?
- Is there heteroscedasticity for premetrological variation, and if it exists, can it be evaluated?
• When a clinical laboratory has produced biological reference values according to IFCC recommendation, should systematic error be referred to as the conventional true value of the calibrators used during the production of reference values?

Although some of the most relevant elements contributing to uncertainty can potentially be evaluated in clinical laboratories, the effort required to undertake such an endeavor might be so great that it will be difficult to bring into general use the uncertainty of patients’ results.

References

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Fluorogenic Probes to Detect the A_{-444}C Transversion in the Leukotriene C4 Synthase Promoter

To the Editor: Adverse hypersensitivity reactions to aspirin and other nonsteroidal anti-inflammatory drugs (1) resemble pseudoallergic reactions with bronchospasm, chronic rhinoconjunctivitis, nasal polyps, urticaria, angioedema, life-threatening bronchospasm, and anaphylactic shock (2). Up to 25% of hospital admissions for acute asthma may be caused by nonsteroidal anti-inflammatory drug ingestion. In aspirin-induced asthma, cysteine-leukotriene release into airways (3, 4) is associated with increased concentrations of leukotriene C4 synthase, the enzyme that forms leukotriene C4 [for review, see Ref. (5)]. A polymorphism in the promoter region of the gene for leukotriene C4 synthase predisposes to disease (4). The A_{-444}C transversion creates a new MspI restriction site.

PCR-restriction fragment length polymorphism analysis has been used to detect this polymorphism (4). This method is time-consuming and requires multiple manual steps. We used rapid-cycle PCR combined with real-time fluorescence monitoring and fluorescent probe melting point analysis to detect the polymorphism (6) in 1 h.

Genomic DNA from 300 patients was extracted from whole blood or serum (n = 20). To confirm the LightCycler genotyping results, DNA samples (n = 60) were analyzed by PCR-restriction fragment length polymorphism analysis as described (4). The two methods were in complete agreement. PCR was performed in a reaction volume of 10 μL with 1 μL of DNA in the presence of primers (0.5 μmol/L; sense, 5’-TC-CATTCTGAAGCAllele-specific, 5’-ACACACTTCTGCTTGTCCGTT3’ with reaction buffer (LightCycler DNA Master Hybridization Probes 10× buffer, 1.75 mmol/L; Roche Molecular Biochemicals) and the probes (0.2 μmol/L). The detection probe 5’-ACCTTATCTGTTCCCCCTCCCAT-3’ was labeled at the 3’ end with fluorescein; the anchor probe 5’-CCAGGCCTCGCCGCTAACTCCTCC-3’ was labeled with LC Red 640 at its 5’ end and modified at the 3’ end by phosphorylation to block extension.

The amplification and melting curve analyses were performed in a LightCycler device and included initial denaturation at 94 °C for 120 s, followed by 60 cycles of denaturation (94 °C for 0 s, with a temperature transition rate of 20 °C/s); annealing (57 °C for 10 s; transition rate, 20 °C/s), which allowed both primer and probe annealing; and extension (72 °C for 13 s; transition rate, 20 °C/s). After amplification, we recorded the melting curve by cooling the reaction mixture to 54 °C for 10 s and then by slowly raising the temperature to 80 °C at 0.1 °C/s. The fluorescence signal (F) was continuously monitored during the temperature ramp and then plotted against the temperature (T) to obtain melting curves for each sample. The melting curves were subsequently converted to derivative melting curves [−(dF/dT)] vs T). The melting curves of the AA, AC, and CC genotypes of the LTC gene are shown in Fig. 1. The melting peaks of samples homozygous for the A allele were at 64.4 °C (range, 63.7–64.9 °C), whereas in samples homozygous for the C allele, the melting peak was 59.0 °C (range, 58.7–59.4 °C). The heterozygous samples produced both peaks. Repeated analysis (10 times) of three samples with different genotypes (AA, AC, CC) showed no misclassifications.