Uncertainty of measurement in clinical microbiology

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An important part of the activity in a clinical microbiology laboratory is the measurement of quantities related to concentrations of microorganisms, antibodies, nucleic acids, etc. When measuring a microbiologic quantity random and systematic errors can act together on the result producing an error of measurement and generating a doubt - uncertainty - about the true value of the measured quantity. International scientific organizations, keeping in mind these facts, have developed the concept of uncertainty of measurement (1,2). The importance of this concept is increasing in all fields of health sciences (3-5). For this reason, it is important to clarify the concept and show the practical way to bring estimate the uncertainty of patients' results.

Uncertainty of measurement is a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to 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):

1. Standard uncertainty (u) is the standard deviation that denotes the uncertainty of the result of a single measurement.

2. Combined standard uncertainty (uc) 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.

3. Expanded uncertainty (U) is the statistic defining the interval within which the value of the measured quantity 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-a) desired. If k = 2, then 1-a » 0,95; if k = 2,6, then 1-a » 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 requires also evaluation of the doubt attached to its value. The common opinion of these bodies is that clinical laboratories should supply information about the uncer-tainty of their results of measurement, when applicable

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 using assumed probability distribution based on experience or other available information, termed type B standard uncertainty (2,6).

In general, in clinical microbiology the most relevant elements that can contribute to uncertainty for a given measurement procedure are:

1. incomplete definition of the particular quantity under measurement (specially for antigen and antibodies); 2. pre-analytical variation; 3. uncertainty related to calibration processes; 4. inappropriate calibration function used by an analyzer; 5. interference; 6. imprecision; 7. rounding of results.

All these sources of uncertainty do not apply in all cases; for each measurement procedure is necessary to identify which of these sources should be taken into account.

In the following examples the estimation of the uncertainty of measurement of two typical microbiological quantities is presented step-by-step.

Example 1: Measurement of the number concentration of bacteria in urine.

In this example, the number concentration (num.c.) of bacteria in urine (U) is measured by direct visual counting of the colonies on the culture medium of a Petri dish produced by 0,001mL of urine inoculated with an appropriate calibrated loop. The result is obtained multiplying the number of colonies counted on the Petri dish per 106. Let a patient's result [according to internationally recommended presentation (5, 7)] be:

$$U\text{—Bacteria; num.c.} = 100 \bullet 10^6/L$$

Assuming that urine has been appropriately collected and processed, the relevant components of uncertainty that should be taken into account are the calibration of the loop and the imprecision of sampling the urine specimen.

Calibration of the loop. - The relative standard uncertainty of the volume dispensed by the calibrated loop declared by the manufacturer is 5 %. This relative standard

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uncertainty applied to the patient's result (100 • 106/L) expressed as standard deviation, that is to say standard uncertainty, is 5 •106/L.

Imprecision of sampling. - If repeated samples of a suspension of bacteria in urine are taken, the different numbers of bacteria in these samples follow a Poisson distribution8, in which the median and the variance have the same value. Consequently, assuming that the number of colonies corresponds to the number of bacteria in the urine dispensed by the calibrated loop, the standard uncertainty of the counted number of colonies is equal to the square root of that number. In our case, as the counted number of colonies is 100, the standard uncertainty due to the sampling process is equal to 10, corresponding to a number concentration of 10•106/L.

When the standard uncertainties of every component of uncertainty have been estimated, the combined standard uncertainty (uc) due to all these components may be estimated:

$$uc = [(5 \bullet 10^6/L)^2 + (10 \bullet 10^6/L)\ 2]0,5 = 11,2 \bullet 10^6/L$$

Finally, we will estimate the expanded uncertainty (U) with a confidence level 1-a » 0.95 multiplying the combined standard uncertainty by a coverage factor (k) equal to 2:

$$U = uc \bullet k = (11,2 \bullet 10^6/L) \bullet 2 = 22,4 \bullet 10^6/L$$

Thus, the complete patient's result will be:

$$U\text{—Bacteria; num.c.} = (100 \pm 22) \bullet 10^6/L$$

Example 2: Measurement of the number concentration of human immunodeficiency virus 1 in plasma

In this example the number concentration (num.c.) of human immunodeficiency virus 1 in plasma (P) [usually called viral load] is known by means the measurement of the amount of viral RNA in the sample, using a sandwich nucleic acid hybridization and chemiluminiscence procedure with six calibrators.

Let a patient's result [according to internationally recommended presentation (5,7)] be:

$$P - \text{Human immunodeficiency virus 1 (RNA); num.c.} = 35663 \bullet 10^3/L$$

The relevant components of uncertainty of measurement that should be taken into account are the uncertainty of the values assigned to calibrators and the day-to-day imprecision.

Uncertainty of the values assigned to calibrators. - The relative expanded uncertainty of every one of the six calibrators declared (after request) by the manufacturer is 3%; thus, assuming that the coverage factor used is 2, the relative standard uncertainty of every calibrator is 1.5 %.

When a measurement procedure needs several calibrators, the uncertainty of measurement due to the complete set of calibrators is given (approximately) by the following formula (6):

$$uc\ rel. \text{ » } (urel.12+ urel.2\ 2+ ..... + urel.n\ 2)0,5/n$$

where uc rel. is the relative combined standard uncertainty due to the entire set of calibrators, u rel. is the relative standard uncertainty of each calibrator and n is the number of calibrators used. In this example the above formula gives a relative combined standard uncertainty approximately equal to 0,6 %, which applied to the measurement result (35663• 10^3/L) corresponds to an standard uncertainty equal to 214,0 • 10^3/L.

Day-to-day imprecision. - In this example, data from internal quality control show a day-to-day coefficient of variation equal to 20% within the measurement range of the measurement procedure. This imprecision applied to the patient's result (35663 • 10^3/L) expressed as standard deviation, or standard uncertainty, is 7132,6 • 10^3/L.

When the standard uncertainties of every uncertainty component have been estimated, the combined standard uncertainty (uc) due to all these components may be estimated:

$$uc = [(214,0 \bullet 10^3/L)^2 + (7132,6 \bullet 10^3/L)^2]0,5 = 7135,8 \bullet 10^3/L$$

Finally, we will estimate the expanded uncertainty (U) with a confidence level 1-a » 0.95 multiplying the combined standard uncertainty by a coverage factor (k) equal to 2:

$$U = uc \bullet k = (7135,8 \bullet 10^3/L) \bullet 2 = 14271,6 \bullet 10^3/L$$

Thus, the complete patient's result, after rounding the value of the expanded uncertainty as is usually done for the measurement result, will be:

$$P - \text{Human immunodeficiency virus 1 (RNA); num.c.} = (35663 \bullet 14272) \bullet 10^3/L$$

From the above examples it may be appreciated that the addition to the results of the corresponding expanded uncertainty makes these type of measurements more scientifically rigorous, and allows a more objective interpretation of consecutive results when monitoring a patient. Whether or no the uncertainties estimated in these examples are medically relevant is out of the scope of this article.

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Errata Corrige

Articolo "Valutaizone comparativa di due contaglobuli automatici" di Simona Brambilla, Fabio Campielli, Irene Moraschinelli e Vittori Grazioli", Biochimica Clinica 2002; 28(2): 86-90

1. La Tabella 4 (pagina 89) è errata. La tabella corretta è la seguente:

Tabella 4

Confronto tra le differenze percentuali medie, la varibilità biologica inter-individuale (Vb) e la differenza critica (Dcr)

Parametro ematologico	Differenza % (Beckman-Abbott)		Vb	Dcr
	Questo lavoro	VEQ	%	%
Leucociti, 10^9/L	-2,24	+9,51	10,9-11,7	33,8-36,2
Eritrociti, 10^{12}/L	-3,28	-0,73	2,4-3,2	7,4-9,9
Emoglobina, g/dL	+0,88	-0,88	2,1-2,8	6,5-8,7
MCV, fl	+1,13	-1,93	1,3-1,4	4,0-4,3
Piastrine, 10^9/L	+3,17	-11,2	7,0-11,9	21,7-36,8
RDW, %	+13,5	n.d.	n.d.	n.d.
Ematocrito, %	-2,29	-2.62	2,1-2,8	6,5-8,7

n.d. non disponibile

2. Il nome di uno degli Autori è errato:
leggasi Cambielli anzichè Campielli