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Laboratory Management

A Model for an Uncertainty Budget for Preanalytical Variables in Clinical Chemistry Analyses

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Background: **We sought a practical method to calculate preanalytical uncertainties. In clinical chemistry measurements, the combined preanalytical uncertainty is a function of the magnitude and probability distribution of the different uncertainty sources and the number of such sources.**

Methods: **Results from an optimal practice for handling of the blood samples (termed the standard method) were compared with alternative methods that deviate from the standard method but are used in current practice. For categorically distributed uncertainty sources (e.g., use of different kinds of blood tubes), alternative treatments were modeled discretely using a known probability distribution for each alternative. For continuously distributed sources (e.g., clotting time), we assumed a rectangular distribution. We calculated the expectation, variance, and SD of differences between results from current practice and the standard method. We tabulated uncertainty budgets for the differences between current practice and the standard method for each uncertainty source. The expected individual biases and variances were summed to obtain the combined expected bias and variance.**

Results: **The combined expected bias (SD) for glucose was −0.15 (0.130) mmol/L, with prolonged clotting time giving the greatest contribution. The combined expected bias (SD) for calcium was −0.011 (0.0182) mmol/L, for magnesium 0.006 (0.026) mmol/L, and for creatinine 0.5 (1.81) μmol/L.**

Conclusion: **By comparing a standard method for preanalytical sample handling to alternative methods used in current practice, and considering the distribution of alternative methods, our modeling approach allows the development of an uncertainty budget for preanalytical variables in clinical chemistry analyses.**

In clinical chemistry measurements, the uncertainty in patient results includes both preanalytical and analytical variation, as well as intraindividual biological variation. Preanalytical variables such as sample collection, handling, transport, and storage influence patient results before measurement *(1)*. When standardized procedures are followed, preanalytical variation may be minimized *(2)* and the number of errors in the preanalytical phase decreased *(3)*. Traditionally, laboratories have focused on the uncertainty in the analytical process, but characterization of uncertainty should include the whole process from phlebotomy until reporting of results *(4)*. With all uncertainties quantified and presented together in tabular form as an uncertainty budget, the laboratory will have a tool to identify important uncertainty sources.

The combined uncertainty is a function of the magnitude and probability distribution of the different uncertainty sources and the number of such sources. The uncertainty can be reduced, and laboratory quality improved, by focusing on the sources that contribute most to the combined uncertainty.

The *Guide to Expression of Uncertainty in Measurement* (GUM)[4] is the internationally accepted technique of expressing uncertainty in measurement *(5)*. GUM established general rules for evaluating and expressing uncertainty across a broad spectrum of measurements. GUM expresses the components of uncertainty as standard uncertainties type A and B. Type A evaluation of uncertainty is based on the statistical analysis of series of observations, whereas type B evaluation uses other means

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[4] Nonstandard abbreviations: GUM, *Guide to Expression of Uncertainty in Measurement*; SST, serum separation tube.

(5). Several guidelines explain how the concepts in GUM can be applied in chemical measurement *(6–8)*, but we have not been able to find any comprehensive method on how to estimate preanalytical uncertainty. Laboratories working toward accreditation by the International Standards Organization 15189 standard are asked to determine the uncertainty of results where relevant and possible *(9)*.

The aim of this study was to develop a practical tool for estimating preanalytical uncertainty in clinical chemistry analyses. We present a model for an uncertainty budget that includes both continuously and categorically distributed uncertainty sources. In the examples presented, we studied glucose, calcium, magnesium, and creatinine. We dealt with the preanalytical uncertainty added to patient results by the use of different kinds of blood tubes and instruments, variations in clotting time and centrifugal force, and delays in measurement.

Materials and Methods

SAMPLE HANDLING AND ANALYSIS

After we obtained informed consent in accordance with the Helsinki Declaration, we performed phlebotomy in accordance with a standardized procedure *(10)*. Additional samples needed for our study were collected from consenting hospitalized patients in the course of routine blood collections for tests already ordered by the patients' physicians.

Blood samples were collected into Vacutainer Tubes (Becton Dickinson): glass serum separation tubes (SSTs), and plastic tubes (SST II Plus), both with gel for serum separation, and plain glass tubes without additive. The tubes were centrifuged for 10 min in swing-out centrifuges (Jouan GR412) at 20 °C.

After separating the serum from the gel or the clot, we analyzed paired samples within 4 h of collection on Roche Modular Analytics SWA instruments by photometric methods (Roche Diagnostics GmbH): glucose hexokinase (glucose), *o*-cresolphthalein (calcium), xylidyl blue (magnesium), and Jaffe (creatinine). Some serum samples were frozen at −80 °C before measurement. For magnesium, we excluded samples that were hemolyzed (based on visual inspection).

THE MODEL

We defined the recommended method in our laboratory for handling blood samples as the standard method (Fig. 1): (*a*) collection of blood into glass SSTs, (*b*) 45-min clotting time, (*c*) centrifugation at 1300*g*, (*d*) fresh serum analysis within 4 h of collection, and (*e*) use of Roche Modular I.

Specified alternative methods at our laboratory deviate from the standard method (Fig. 1, maximum deviation shown) but are within current practice: (*a*) collection of blood into SST II Plus or plain tubes, (*b*) 2-h clotting time, (*c*) centrifugation at 2350*g*, (*d*) 48-h storage of serum, and (*e*) use of Roche Modular II. The 2 Modular instruments (I and II) are in the same room and use identical reagents and measurement methods.

We modeled the current practice (Fig. 1) as collecting blood into glass SSTs with probability $P = 0.8$, SST II Plus tubes $P = 0.1$, and plain tubes $P = 0.1$. We modeled clotting time, centrifugal force, and storage time by rectangular distributions and assumed the probability of analyzing the serum on Modular I to be $P = 0.5$ and on Modular II $P = 0.5$. These probability distributions were compatible with actual use in our laboratory. The sum of probabilities for each uncertainty source was $P = 1$.

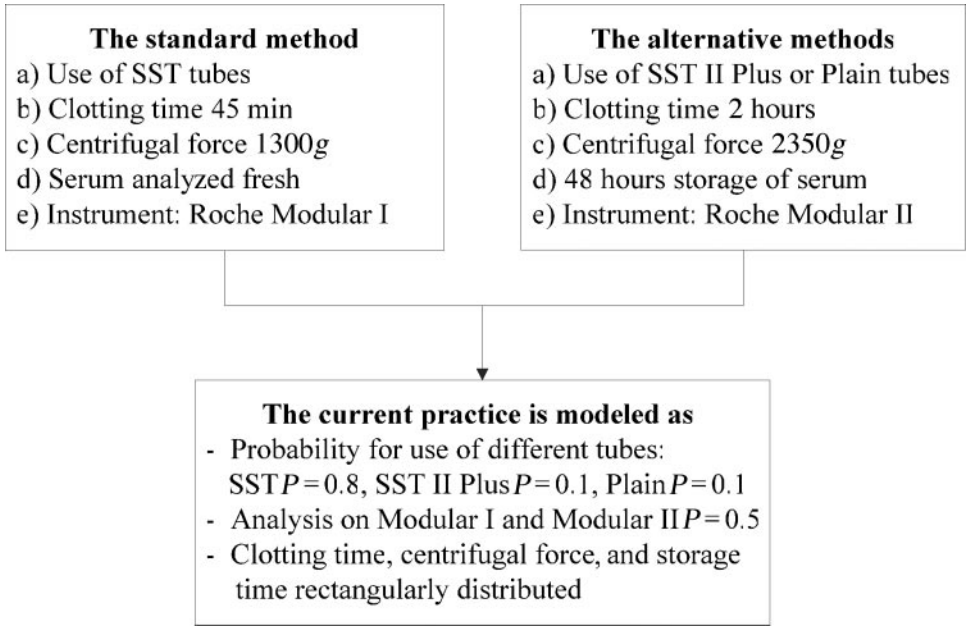

Fig. 1. Description of the standard method, the alternative methods, and current practice.

PREANALYTICAL STUDIES

Each of the uncertainty sources (*a*–*e*) was studied separately by paired observations between the alternative method and the standard method.

(*a*) *Different blood tubes.* One SST (standard method) and 1 plain tube (alternative method) were collected from each patient (n = 33), and 1 SST (standard method) and 1 SST II Plus tube (alternative method) were collected from each patient (n = 34). All pairs of tubes had equal clotting time (between 45 and 120 min) and were centrifuged at 1300*g*, except that the SST II Plus tubes were centrifuged at 1850*g* as recommended.

(*b*) *Clotting time.* Two SSTs were collected from each patient (n = 45). One of the paired SSTs was centrifuged as recommended after 40–70 min clotting time (standard method) and the other after 120–150 min (alternative method).

(*c*) *Centrifugal force.* Two SSTs were collected from each patient (n = 28). One of the paired SSTs was centrifuged at 1300*g* (standard method) and the other at 2350*g* (alternative method) after equal clotting time (between 45 and 70 min).

(*d*) *Storage time.* Two SSTs were collected from each patient (n = 31). All pairs of tubes had equal clotting time (between 45 and 120 min) and were centrifuged at 1300*g*. From one of the paired SST tubes, 500 μL serum was frozen at −80 °C within 4 h (standard method). The other of the paired tubes was left at room temperature for 48 h, and then 500 μL serum from this tube was frozen at −80 °C (alternative method).

(*e*) *Different instruments.* Aliquots of serum samples from routine collection (~500 total) were analyzed 1 per day at equal time points on both Modular I (standard method) and Modular II (alternative method).

STATISTICAL ANALYSIS

The general model for the specific situation presented above (uncertainty sources *a*–*e*) was as follows. We specified a standard (recommended) method of preanalytical treatment of samples for each source of uncertainty. Also, for each source, we specified alternative methods of treatment, which were within clinical practice. We modeled discrete and continuous uncertainty sources separately. For a discrete uncertainty source, we defined a number of alternative treatments, together with their probabilities in current practice. For a continuous uncertainty source, we assumed that the actual treatment was within an interval, with the standard treatment at one end and a maximally deviant treatment, still within current practice, at the other. The estimates were based on paired data for each source. For discrete sources, we collected paired data for standard treatment and each of the alternative treatments. For a continuous source, we collected paired data for the standard source and the maximally deviant treatment. The paired samples were treated the same way, except for the uncertainty source being tested.

We focused on the difference D between current practice and standard treatment. For each source the mean $E(D)$ and SD, called $SD(D)$ [by definition the square root of the variance $Var(D)$], were estimated, based on the paired data. Finally, the mean and SD of the difference D between current practice and standard treatment were estimated with all sources of uncertainty put together. At this final stage, we assumed that, for each source, the difference D had the same probability distribution irrespective of the treatment used for the other sources, within current practice, with independent differences for different sources. Under these assumptions, as detailed in the Appendix (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue7), the mean and variance of D were simply the sum of the means and variances for each individual source *(11)*.

For a laboratory conforming with the GUM guidelines there is no bias; therefore, the mean $ED = 0$ for all sources. Because this objective is not generally fulfilled in actual practice in the preanalytical steps, we included the possibility of bias in our model.

In the following, we present modeling scenarios for the case of a discrete source first, and then the case of a continuous source. We introduce notation and state formulas for computing the (estimated) means and variances of the difference D in each case. Although these formulas may seem complicated, they are straightforward to program, and Microsoft Excel sheets (with references to formulas below) have been prepared that alleviate the burden of computation. See Uncertainty Calculations in the online Data Supplement.

For a discrete source, the standard treatment is called treatment number 1, together with alternative treatments 2, 3, . . . , n. Assume a known probability distribution for the use of each of the n treatments in current practice. This distribution should be estimated from frequency data on the use of each treatment within the laboratory. Means of the difference D are, as stated above, estimated from paired data.

Notation:

- $p_1, p_2, \ldots, p_n$: Probabilities for each treatment (method 1 is standard treatment)
- $e_1 = 0, e_2, \ldots, e_n$: Mean $E(D)$ of the difference D for each treatment
- $v_1 = 0, v_2, \ldots, v_n$: Variance $Var(D)$ of the difference D for each treatment.

Result 1 (for proof, see Appendix in the online Data Supplement): The mean $E(D)$ and variance $Var(D)$ in current practice for the categorically distributed source in question are as follows *(12)*:

$$E(D) = p_2e_2 + p_3e_3 + \ldots + p_ne_n \quad (1)$$

$$Var(D) = p_2e_2^2 + p_3e_3^2 + \ldots + p_ne_n^2 - [p_2e_2 + p_3e_3 + \ldots + p_ne_n]^2 + p_2v_2 + p_3v_3 + \ldots + p_nv_n. \quad (2)$$

For a continuous source, we assume that the treatment T in current practice forms a continuum, with the standard treatment at one end ($T = 0$) and a maximally distant alternative treatment ($T = 1$) at the other end. It is assumed that the treatment T actually used is rectangularly distributed. Also, it is assumed that the mean and SD for the difference D are linear functions of the treatment actually used, with slopes α and β, respectively. More precisely:

$$E(D \mid T = t) = \alpha t, \text{ for } t = 0 \text{ (standard treatment) to } t = 1 \text{ (maximally distant treatment)} \quad (3)$$

$$SD(D \mid T = t) = \beta t, \text{ for } t = 0 \text{ (standard treatment) to } t = 1 \text{ (maximally distant treatment).} \quad (4)$$

Result 2 (for proof, see Appendix in the online Data Supplement): The mean $E(D)$ and variance $Var(D)$ in current practice for the continuously distributed source in question are as follows *(12)*:

$$E(D) = \alpha/2 \quad (5)$$

$$Var(D) = \alpha^2/12 + \beta^2/3. \quad (6)$$

Because we were interested in the variance due to preanalytical treatment ($Var(D)$), we adjusted the variance of differences actually measured, $Var(D_m)$, between alternative methods and the standard method for analytical variation for each treatment, as detailed in the Appendix *(11)*.

We estimated the analytical variation by analyzing different patient samples (n = 34) at the same concentration range as the samples in the different preanalytical studies. The serum samples were analyzed 1 per day in duplicate on Modular I for the calculation of the within-run SD and reanalyzed the next day for the calculation of the between-run SD. The formula used to calculate the SDs was as follows *(13)*:

$$SD = \sqrt{\frac{\Sigma d^2}{2n}}, \quad (7)$$

where d is the difference between the duplicate results and n is the number of duplicate measurements. The within-run SD was used to adjust the variance of differences in the uncertainty sources (*a*–*d*), and the between-run SD to adjust the variance of differences in uncertainty source (*e*; different instruments).

Results

Table 1 displays for each component and uncertainty source the mean, variance, and SD of differences from paired observations between alternative methods and the standard method, P values from paired t-tests, and the concentration range of the data. Paired t-tests showed significant differences ($P < 0.05$) for plain tubes vs SSTs (creatinine), clotting time (glucose and calcium), storage time (glucose and creatinine), and Modular II vs I (glucose and creatinine).

Preanalytical uncertainty budgets are presented in Table 2 for each uncertainty source as the expectation, variance, and SD of differences between current practice and the standard method, and the sum of the expected individual biases and variances.

We estimated the within-run SD (SD_{wr}) and the between-run SD (SD_{br}) as follows (SD_{wr}, SD_{br}, concentration range of the samples): glucose (0.05 mmol/L, 0.07 mmol/L, 3.4–11.7 mmol/L), calcium (0.019 mmol/L, 0.033 mmol/L, 1.97–2.52 mmol/L), magnesium (0.008 mmol/L, 0.010 mmol/L, 0.68–1.03 mmol/L), and creatinine (1.2 μmol/L, 1.9 μmol/L, 51–174 μmol/L).

Discussion

In the preanalytical phase of clinical chemistry analyses, many sources may contribute to the uncertainty of the result. An uncertainty budget can be used to find the sources that contribute most to the combined uncertainty *(4)* and is therefore an excellent tool for optimizing the quality of measurements.

In the uncertainty budget model presented here, we define a preferred procedure for handling the blood samples (the standard method) and various deviations from this procedure (alternative methods) that may occur in clinical practice. In practice, the alternative methods are used to a variable extent. In the model, we therefore include a probability distribution of the use of the alternative methods, and Table 2 shows the biases, variances, and SDs estimated from current practice, based on the mean, variance, and SD of differences in results obtained by the alternative vs the standard method (Table 1). The bias in current results will increase with increasing use of the alternative method. However, the combined bias does not necessarily increase with increasing number of uncertainty sources, since biases to some extent may neutralize each other. In contrast, when adding variances, the combined variance increases, but minor contributions have little influence on the combined variance. In fact, the combined SD (square root of the combined variance) is usually a more interpretable quantity than variance, and it is only slightly influenced by even a fairly high number of small contributions.

The budget as introduced here can be specified from the routine work in the individual laboratory. An unlimited number of uncertainty sources, both categorical and continuous, can be included. The Microsoft Excel sheets developed by the authors are intended to make the calculations feasible in actual laboratory use.

As an example of using the model, if the clotting time was prolonged from 45 min (standard method) to 2 h (alternative method), the result of glucose was changed by, on average, −0.182 mmol/L (SD 0.102 mmol/L; Table 1). The expected

Table 1. Results from paired observations between alternative methods and the standard method.

Uncertainty source	n	Mean (95% CI)[a]	Variance[b]	SD[c]	*P*[d]	Range[e]
A. Glucose, mmol/L						
Plain tubes vs SSTs	33	<0.001 (−0.031 to 0.031)	0.0076	0.087	1.00	4.2–11.1
SST II Plus tubes vs SSTs	34	−0.029 (−0.061 to 0.002)	0.0083	0.091	0.07	3.0–11.2
Clotting time	44	−0.182 (−0.213 to −0.151)	0.0104	0.102	<0.001	3.6–13.8
Centrifugal force	27	−0.015 (−0.050 to 0.021)	0.0081	0.090	0.40	3.5–6.7
Storage time	30	−0.120 (−0.167 to −0.067)	0.0180	0.134	<0.001	3.9–12.4
Modular II vs I	478	0.017 (0.005 to 0.030)	0.0197	0.140	0.007	2.7–13.6
B. Calcium, mmol/L						
Plain tubes vs SSTs	33	−0.003 (−0.014 to 0.008)	0.0010	0.032	0.55	1.75–2.48
SST II Plus tubes vs SSTs	34	−0.001 (−0.010 to 0.008)	0.0007	0.026	0.85	1.98–2.47
Clotting time	44	−0.019 (−0.028 to −0.011)	0.0008	0.028	<0.001	1.91–2.45
Centrifugal force	28	−0.005 (−0.016 to 0.006)	0.0008	0.028	0.40	1.90–2.60
Storage time	31	0.004 (−0.007 to 0.015)	0.0010	0.032	0.49	1.95–2.41
Modular II vs I	494	−0.002 (−0.006 to 0.002)	0.0024	0.049	0.35	1.65–2.75
C. Magnesium, mmol/L						
Plain tubes vs SSTs	32	0.001 (−0.011 to 0.013)	0.0012	0.035	0.92	0.57–1.04
SST II Plus tubes vs SSTs	34	0.014 (−0.002 to 0.030)	0.0021	0.046	0.08	0.61–1.12
Clotting time	39	<0.001 (−0.005 to 0.005)	0.0002	0.014	0.91	0.64–1.18
Centrifugal force	28	0.001 (−0.004 to 0.007)	0.0002	0.014	0.61	0.50–0.94
Storage time	31	0.006 (−0.002 to 0.013)	0.0004	0.020	0.12	0.56–1.00
Modular II vs I	494	0.002 (−0.0005 to 0.004)	0.0006	0.024	0.13	0.52–1.32
D. Creatinine, μmol/L						
Plain tubes vs SSTs	32	−1.6 (−2.5 to −0.6)	7.4	2.72	0.003	43–131
SST II Plus tubes vs SSTs	34	−0.1 (−1.1 to 0.9)	7.8	2.79	0.85	54–181
Clotting time	42	−0.05 (−0.6 to 0.5)	2.9	1.70	0.86	44–145
Centrifugal force	27	−0.5 (−1.3 to 0.3)	4.0	2.00	0.23	38–227
Storage time	31	2.1 (1.2 to 3.0)	6.8	2.61	<0.001	51–129
Modular II vs I	483	−0.3 (−0.5 to −0.1)	6.3	2.51	0.01	51–164

[a] Mean of differences (95% CI for the mean).
[b] Variance of the differences.
[c] SD of differences.
[d] *P* value from paired *t*-test (one sample *t*-test for comparison of the mean against 0).
[e] The lowest and highest values of the patient results.

bias (current practice vs standard method) was −0.091 mmol/L (SD 0.071 mmol/L; Table 2).

The uncertainty budget presented in Table 2 shows that of the uncertainty sources included, prolonged clotting time for glucose gave the greatest contribution to the combined bias of −0.15 mmol/L. Naturally, we found larger deviations between the alternative methods (maximum deviation) and the standard method presented in Table 1 than between current practice and the standard method in Table 2.

The recommended clotting time before centrifugation is 30 to 60 min, and serum should be separated from the erythrocytes as soon as possible, or within 2 h after the phlebotomy *(14)*. In agreement with other studies, the concentration of glucose decreased with prolonged contact with the clot, probably due to glycolysis *(1)*, whereas

Table 2. The preanalytical uncertainty budget shows the expected bias, variance, and SD of the differences between current practice and the standard method for each uncertainty source and the sum of the expected biases and variances.

Uncertainty sources	Glucose, mmol/L			Calcium, mmol/L			Magnesium, mmol/L			Creatinine, μmol/L		
	Bias	Variance	SD	Bias	Variance	SD	Bias	Variance	SD	Bias	Variance	SD
Different blood tubes	−0.003	0.001	0.032	−0.0004	0.00003	0.0055	0.001	0.00032	0.0179	−0.2	1.17	1.08
Clotting time	−0.091	0.005	0.071	−0.010	0.00007	0.0084	<0.001	0.00002	0.0045	−0.02	0.004	0.06
Centrifugal force	−0.008	0.001	0.032	−0.002	0.00004	0.0063	0.001	0.00003	0.0055	−0.2	0.43	0.66
Storage time	−0.058	0.006	0.077	0.002	0.00008	0.0089	0.003	0.00009	0.0095	1.1	1.66	1.29
Different instruments	0.009	0.005	0.071	−0.001	0.00011	0.0105	0.001	0.00020	0.0141	−0.2	0.02	0.14
Combined biases and variances	−0.15	0.017	0.130	−0.011	0.00033	0.0182	0.006	0.00066	0.0257	0.5	3.28	1.81

the change in concentration of creatinine, calcium, and magnesium was minimal *(15)*.

The recommended centrifugal force is 1000*g*–1200*g* for 5–15 min *(14)*. Hemolysis due to extended centrifugal force may be expected *(1)*, but none of the components were affected in our study. Temperature and time of storage may influence the stability of biochemical components in serum. The serum should be stored at 2–8 °C when not analyzed within 8 h after phlebotomy *(14)*. As in other studies, however, we found no bias of clinical interest after 48-h storage at room temperature or use of different blood tubes *(16)*. When using the same lot numbers of reagents and calibrators on the 2 Modular instruments, the biases between the instruments were small.

The combined preanalytical variation for some components has been estimated by Fuentes-Arderiu et al. *(17)* without differentiation of the individual steps. In some studies, the preanalytical uncertainty evaluations were based only on reports in the literature or assumptions *(18, 19)*. As in other studies, our budget does not include error from interferences, although interferences are frequently reported in diagnostic tests *(20)*.

As a limitation, we emphasize that some important model assumptions (although presumably reasonable) and simplifying approximations are not empirically justified at present. These assumptions include linearity of means and SDs for continuous sources of uncertainty and identical distributions of the difference for each source of uncertainty irrespective of the status of the other sources. Work to investigate these assumptions is being planned at our laboratory, and similar scrutiny by other groups is encouraged.

The model can also be applied to prephlebotomy variables. A standard procedure (e.g., sitting 15 min before phlebotomy) and an alternative procedure must be defined. The bias, variance, and SD of the differences resulting from standard vs alternative treatment are then determined, and the probability distribution for use of the alternative method is estimated. The evaluation of preanalytical uncertainties should depend on detailed knowledge of the nature of the component and of the sample handling.

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