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Evaluation of measurement uncertainty in clinical chemistry

Applications to determinations of total concentration of calcium and glucose in human serum

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1. Introduction

This report demonstrates how the measurement uncertainty for two routine clinical chemistry applications can be evaluated. The examples were prepared as an educational aspect of IMEP-17, an interlaboratory comparison organised in collaboration with EQALM[*] and the C-AQ IFCC.[1,†] The evaluation follows the general principles in the Guide to the Expression of Uncertainty in Measurement (GUM),[2] and in the Eurachem/CITAC Guide Quantifying Uncertainty in Analytical Measurement.[3] The approach is also consistent with the requirements of ISO/IEC 17025 General Requirements for the Competence of Calibration and Testing Laboratories,[4] and of ISO/DIS 15189 Quality Management in the Medical Laboratory.[5] Internationally accepted metrology terms[6] as well as sector specific language is used in the report.

According to good laboratory practice, all relevant work should be documented, e.g. in a standard operating procedure. When the measurement uncertainty is evaluated, we assume that the instructions in the procedures are followed and that no gross errors occur.

1.1. Calcium

Calcium is the most abundant mineral element in the human body with about 99 percent in the bones. In addition to skeletal functions, calcium is involved in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Serum calcium levels and hence the body content, are controlled mainly by the two hormones parathyroid hormone (PTH) and calcitonin, and by vitamin D.[7] The expected total concentration of calcium in serum of healthy adults is in the range 2.25-2.65 mmol/L.[8]

1.2. Glucose

The human body maintains blood glucose in a very narrow range mainly by the two pancreas endocrine hormones insulin and glucagon. Blood glucose measurements are used for diagnosis of disorders in carbohydrate metabolism. Hyperglycemia with elevated blood glucose levels is found in diabetes mellitus, and in other disorders, e.g. pancreatitis, thyroid dysfunction, renal failure, and liver disease. Hypoglycemic levels may be found in a variety of conditions, such as insulinoma, hypopituitarism, or insulin-induced hypoglycemia.[9] The expected glucose concentration in fasting samples is 4.2-6.0 mmol/L.[10,11]

1.3. Principles of measurement and instrumentation

1.3.1. Calcium

The total concentration of calcium is determined using a spectrophotometric measurement method. Calcium ions form a violet-coloured complex with *o*-cresolphtalein complexone (*o*-CPC) under alkaline conditions.[12] Additional reagents used in the determination include a non-reactive surfactant (CAPS),[‡] and 8-hydroxyquinoline to prevent interference of magnesium and iron. The quantification is based on measuring the absorbance at 552 nm and 629 nm.

[*] European committee for external Quality Assurance programmes in Laboratory Medicine

[†] Committee for Analytical Quality of the International Federation for Clinical Chemistry and laboratory medicine

[‡] (3-[cyclohexylamino]-1-propanesulfonic acid)

1.3.2. Glucose

The concentration of glucose is determined using a spectrophotometric procedure. Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by NAD^+ to form NADH.[13] The concentration of NADH formed is directly proportional to the glucose concentration. The reagents used in the determination contain a non-reactive (3-[N-morpholino] propanesulfonic acid, ATP, NAD^+, Mg^{2+}, HK (from yeast), G6PDH (of microbial origin), and sodium azide as preservative. The quantification is based on measuring the absorbance at 340 nm.

1.3.3. Instrumentation

A fully automated clinical chemistry diagnostic system has been studied in this example. This system represents instrumentation commonly used in larger medical laboratories (Cobas® Integra, Hitachi, Technicon, Beckman analyzer series, etc.), but neither the typical values nor the standard uncertainties are claimed to be those of any specific diagnostic system. The instrument is equipped for measurements based on one or more of the following principles: spectrophotometry, turbidimetry, fluorometry, and potentiometry using ion- selective electrodes. It has access to numerous different onboard assays covering special areas, such as basic clinical chemistry, specific protein analysis, therapeutic drug monitoring, and drugs of abuse testing with a very high throughput per hour. This type of instrumentation enables handling of more than hundred sample tubes under controlled temperature conditions.

In the spectrophotometric applications studied here, sub-samples between 2 and 10 μL are mixed with reagents to final volumes between 120-240 μL and measured in disposable cuvettes with 5 mm path length. The chemical reactions are thermostated to 37±0.1 °C. The normalized and blank-corrected absorbance signal is directly proportional to the total calcium (or glucose) concentration in the sample. In every absorbance calculation, the instrument automatically performs corrections for the cuvette blank and volume changes at two different time points.

1.4. Scope and limitations of the uncertainty evaluations

Three different cases are studied here. They adhere to different situations where part of, or all steps in a measurement procedure, should be evaluated for uncertainty. The evaluation takes into account pre-analytical, analytical and patient-related uncertainty sources. The examples concern calcium and glucose but the uncertainty for other analytes may indeed be evaluated in the same or similar way.

Case 1. Only uncertainty sources in the analytical phase are considered. This is the case, e.g. when the analysis is performed on a sample from another laboratory, or from an organiser of an external quality assessment (EQA) scheme. We assume that the analysis is done as soon as possible after that the sample has arrived and that the laboratory has no responsibility for how the sample is taken, prepared and transported. The tube containing the serum is placed in the instrument. In both applications, a defined volume of the sample is mixed with reagent(s) and diluent(s), and then measured. The uncertainty sources in this case are associated with the measured absorbances and the calibrator values. This case also takes into account an allowed drift in sensitivity. For glucose, the evaluation exemplifies a situation where the concentration exceeds the specified measuring range and a ten-fold dilution is performed before the measurement. For calcium, we also have information about the calibrator, which might cause a matrix effect in certain measurement systems.

Case 2. In this case also the pre-analytical work (sampling, sample pretreatment and storage) is considered. A specimen (whole blood) is collected from the patient and dispensed into a tube

containing the appropriate reagents to prepare the sample. The serum is separated from the blood cells by centrifugation and stored in the tube until analyzed. All steps are performed within the same hospital.

Case 3. This evaluation adds the patient-related uncertainty source. This can be relevant, e.g. when the medical doctor is using the result in his diagnosis or treatment of the patient.

Results from measurements are not perfect, nor the estimation of their uncertainties. Both are based on models. The measurands discussed here are temperature-dependent (volumetric concentrations) but this issue is not discussed, neither in relation to the specification of the measurand nor to as how to take into account the effect from deviations from the reference temperature.

2. The evaluation process

2.1. Introduction

The document from Eurachem/CITAC[3] outlines a four-step procedure for the evaluation. In brief, this means to 1) specify what you intend to measure (the measurand), 2) identify all uncertainty sources, 3) quantify the uncertainty components and convert them to standard uncertainties, and 4) calculate the combined standard uncertainty ("total uncertainty"). It is important that the procedure is followed.

2.2. Step 1: Specification of the measurand

In this step you describe as clearly as possible what you intend to measure (the measurand) and how this is done. The measurands in this study are the total concentration (mmol/L) of calcium and glucose respectively in a human serum sample. It is useful to describe the main steps of the procedure in a simple flowchart (Figure 1).

The uncertainty concept requires a model equation with the relationship between the measurand and everything important that effects it. It is recommended that you start with a simple description and develop it further as more information becomes available. A look into the manufacturers' instrument and method descriptions shows that for both calcium and glucose, the values are calculated from a two-point calibration curve and depend on the absorbance of the sample and calibration solutions, and the concentration of the calibration solutions.

The unknown concentration is described by the following model equation

$$c_{\mathrm{x}} = c_0 + \frac{A_{\mathrm{s}} - A_0}{A_{\mathrm{cal}} - A_0} \cdot (c_{\mathrm{cal}} - c_0)$$

where,

c_x : Total concentration of calcium or glucose in the sample solution [mmol/L]

c_0 : Total concentration of calcium (or glucose) in solution used to establish the zero-point of the calibration curve [mmol/L]

A_s : Normalized and blank-corrected absorbance signal of sample solution in the cuvette [AU]

A_0 : Absorbance signal from reagents [AU]

A_{cal} : Normalized and blank-corrected absorbance signal of calibrator solution in the cuvette [AU]

c_{cal} : Total concentration of calcium (or glucose) in the calibrator [mmol/L]

Everything that appears to the right of the symbol "=" in the equation, is referred to as "input quantities". In addition, there may be other uncertainty sources ("influence quantities"), e.g. temperature variations, that do not appear in the expression but still affect the result.

The calibration process is illustrated in Figure 2. The instrument stores the calibration information and may (automatically) display a picture on a printout with an indication of the sensitivity *F* (slope of the line). *F* is calculated from the measured signals and the concentrations according to:

$$F = \frac{c_{\text{cal}} - c_0}{A_{\text{cal}} - A_0}$$

The manufacturer recommends certain calibration intervals for the applications. It is assumed that these instructions are followed.

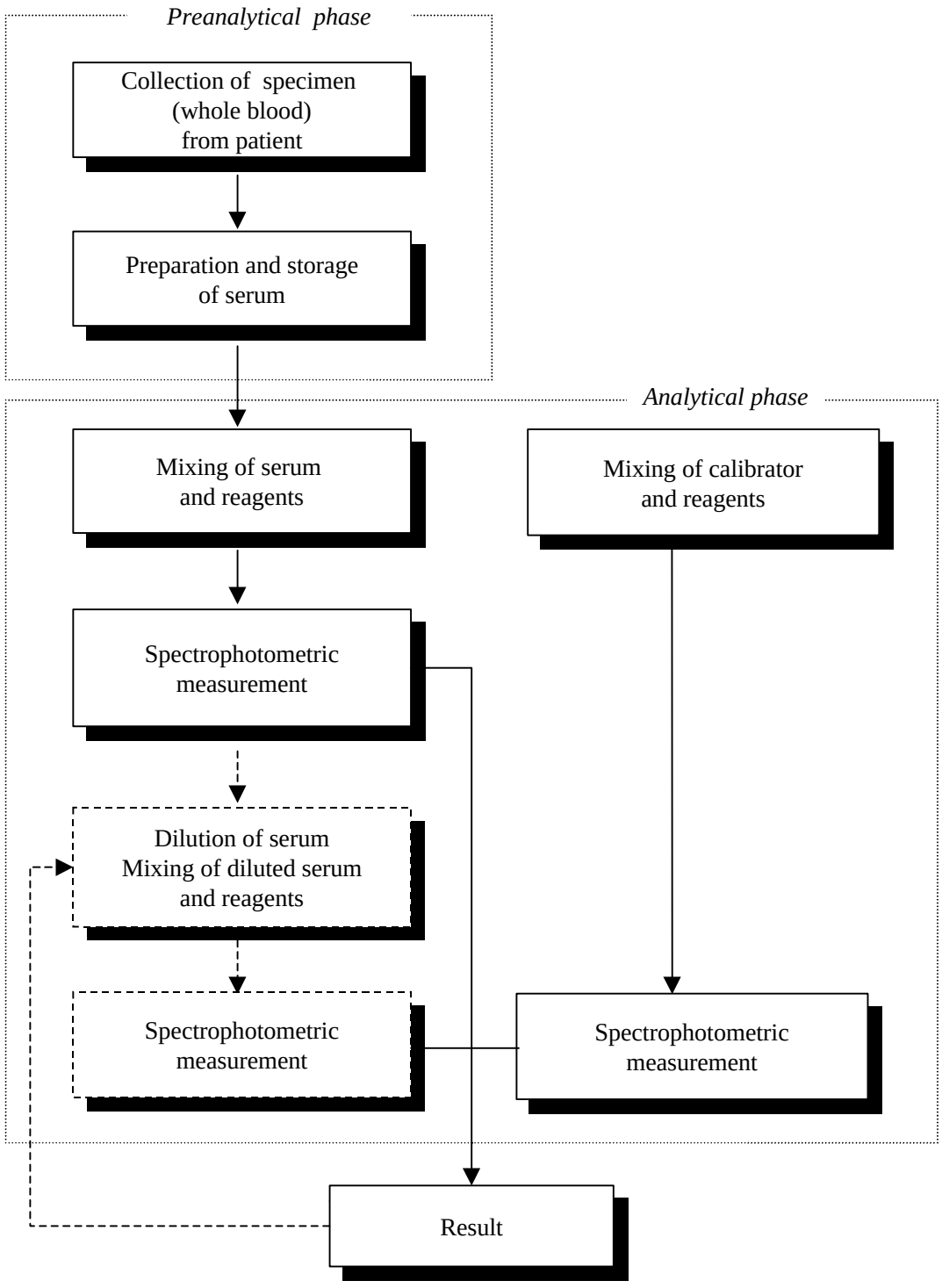

Figure 1. Outline of the measurement procedure. A ready-to-use liquid serum material with assigned values is used for the method calibration. It is assumed that the analytical conditions (volumes and incubation times) in the mixing and measurement steps are the same for the samples and the calibrators. The dilution step is performed when the concentration of the analyte in the sample exceeds the measurement range.

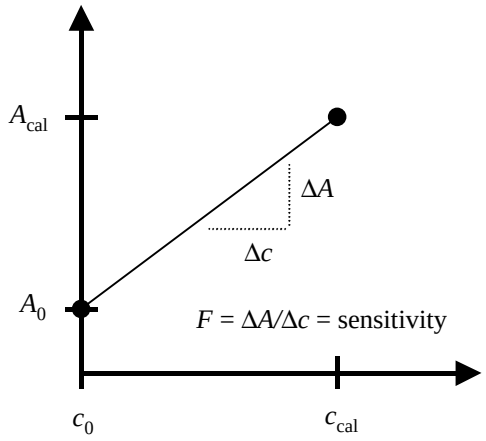

Figure 2. Illustration of a two-point calibration curve. The instrument calculates the sensitivity *F* (slope of the line) from the measured absorbances and the concentrations.

2.3. Step 2: Identifying the uncertainty sources

The aim of this step is to identify and put together a list of all possible uncertainty sources, which affect the value of the measurand. It is convenient to start with the mathematical expression from Step 1. All input quantities in the equation, i.e. $c_0, A_s, A_0, A_{cal}, c_{cal}$, may have an uncertainty associated with their value and will therefore contribute to the total uncertainty. These sources adhere to the analytical phase and below we will describe them more in detail.

2.3.1. Analytical phase

2.3.1.1. Absorbance measurements, A_s, A_0, A_{cal}

The absorbances as measured for the sample and calibration solutions appear as input quantities in the model. Clearly, any variation in these signals contributes to the uncertainty of the calcium or glucose concentration. The measured absorbance readings are stored in the system memory and can be printed out.

2.3.1.2. Calcium and glucose concentrations in calibrators, c_0, c_{cal}

It is common that a manufacturer of an instrument also provides the necessary measurement standards (e.g. calibrators, reference materials). According to the EC directive on *in vitro* diagnostic medical devices, *the traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order.*[14] The measurement standards should be accompanied by a certificate with information about the assigned property values, e.g. c_{cal} and their uncertainties. The lower point of the calibration curve is derived from pure water having no deliberate content of calcium or glucose.

2.3.1.3. Sample dilution, *d*

As indicated in Figure 1, the analytical phase may include a dilution of the sample. This is done by the analyst before the sample is introduced into the instrument, or by the instrument itself. To calculate the concentration in the sample, we need to multiply the result with the dilution factor *d*.

$$d = \frac{V_1 + V_2}{V_1}$$

where V_1 and V_2 are the volumes of sample and diluent (solvent) respectively.

2.3.1.4. Matrix effects, k_{matrix}

It is impossible to have calibrators with exactly the same properties as the patient sample. Even if the concentration of calcium or glucose in the sample and in the calibrator were the same, the concentration of other naturally occurring components may not be. Any difference in composition between sample and calibrator can result in a difference in instrument response and hence an error. The process of freeze-drying and reconstitution of serum is, e.g. known to affect some components. We refer to this as "matrix effects", or that sample and calibrator are not "commutable". We illustrate the possibility of a matrix effect by a factor k_{matrix} in our model.

2.3.1.5. Changes in instrument sensitivity, k_{drift}

Calibration is typically done on a weekly or monthly basis, and control samples measured every six hours. The information from the control samples is fed into a chart as part of the internal quality control procedures. Changes in the instrument sensitivity ("drift") between calibrations are directly proportional to changes in concentration and constitute a source of uncertainty. If measurement(s) on a control sample indicates that the sensitivity has changed and falls outside an accepted interval, additional controls are performed and/or the method(s) is re-calibrated. The stability of the instrument is tested by the manufacturer during installation based on given specifications, and/or by the laboratory during the instrument validation. A factor k_{drift} is introduced in the model to handle the uncertainty associated with the allowed drift.

2.3.2. Pre-analytical phase

2.3.2.1. Specimen collection and sample pretreatment, k_{pre}

During the specimen collection (sampling) and the sample pretreatment there are many possible sources of uncertainty. Examples include:

- contamination and/or losses due to equipment used,
- collection technique and general handling of sample to avoid, e.g. hemolysis,
- time and temperature effects, e.g. during storage
- special instrument settings, e.g. position of tube and speed during centrifugation,
- concentrations of chemicals and reagents, e.g. those present in the tube where the specimen (whole blood) is collected.

Serum should be separated from blood cells as soon as possible: prolonged contact with the clot may cause lower calcium values,[15] and contact with the erythrocytes affects the stability of glucose.[16] The above uncertainty sources are normally investigated during the method development, resulting in a procedure where the effects on the measurand are small. Where possible, the size of the effects should be determined, e.g. by changing the conditions and monitor the effect on the result. Information about these effects can also be found in the scientific literature. This is illustrated in Example A5 in the Eurachem/CITAC Guide.[3] The combined effect of many pre-analytical sources of uncertainty has been investigated.[17] We introduce a term k_{pre} in the mathematical model to indicate this source. The value and uncertainty is estimated in Step 3.

2.3.3. Intra-individual effects due to biological variation, k_{intra}

The concentrations of all clinically relevant components, including calcium and glucose, in the human blood depend on biological and other factors, e.g. diurnal rhytm, diet, stress and sleep. These can be included in the uncertainty evaluations hence simplifying for, e.g. the medical doctor who uses the information in diagnosing and decision-making. We introduce a factor k_{intra} for this intra-individual effect and use values from the literature.[18]

2.3.4. Extending the measurement model

Above we have discussed all parts of the measurement procedure (Figure 1) and highlighted uncertainty sources, which may be important. It is now time to modify our model so that it reflects this new information.

$$c'_x = \left[c_0 + \frac{A_s - A_0}{A_{cal} - A_0} \cdot (c_{cal} - c_0) \right] \cdot d \cdot k_{\text{matrix}} \cdot k_{\text{drift}} \cdot k_{\text{intra}} + k_{\text{pre}}$$

The new uncertainty sources are summarised below.

- c'_x : Total concentration of calcium (c_{Ca}) or glucose (c_{gluc}) in the patient sample [mmol/L]
- d : Factor describing the possible sample dilution either by the analyst or the instrument
- k_{matrix} : Factor describing the contribution from possible matrix effects (difference in commutability of sample and calibrator)
- k_{drift} : Factor describing the contribution from an allowed drift in instrument sensitivity
- k_{pre} : Term describing the contribution from pre-analytical work (losses, contamination etc.) [mmol/L]
- k_{intra} : Factor describing the contribution from the intra-individual biological variation of total calcium (or glucose)

Remark: As indicated in the extended model, it is possible to take into account the above effects by introducing either a (correction) factor or (correction) term in the model. The nominal value (in absence of effects on the result) is 1 (correction factor) and 0 (correction term), but they may have an associated uncertainty.

2.4. Step 3: Quantifying and standardising the uncertainty components

In this step we look at all information we have about the uncertainty sources from Step 2. This information comes from within the laboratory (method development, validation, and new and previous measurements), from manufacturers of instruments and equipment (specifications and test reports), from certificates (e.g. for the calibrators and reagents), and from the scientific literature (e.g. biological variation).

All uncertainty contributions (uncertainty components) should be expressed as so-called standard uncertainties (standard deviations). This requires information or assumptions on how values for the specific quantity are distributed (e.g. normal, rectangular, triangular). References 2 and 3 discuss this in detail. Below we summarise information on all the identified uncertainty components. In each case a value and an uncertainty are indicated, and there is information about the source of information and how it was evaluated (Type A: Statistical methods, or Type B: Other methods). There are also constants appearing in the model.

2.4.1. Quantifying uncertainty components in the calcium determination

c_0:
Constant
Value: 0 mmol/L

A blank solution (distilled/deionized water) is used as the second calibrator.

A_s:
Type A
Method of observation: Direct
Number of observations: 1

No.	Observation
1	0.2936

Arithmetic Mean: 0.29360 AU
Pooled Standard Deviation: 0.99%
Standard Uncertainty: $2.91 \cdot 10^{-3}$ AU
Pooled Degrees of Freedom: 19

The observed absorbance value comes from the measurement of the sample. The standard uncertainty was estimated during the method validation by twenty repeated measurements.

A_0:
Type A
Method of observation: Direct
Number of observations: 2

No.	Observation
1	0.0609
2	0.0605

Arithmetic Mean: 0.060700 AU
Pooled Standard Deviation: 2%
Standard Uncertainty: $838 \cdot 10^{-6}$ AU
Pooled Degrees of Freedom: 19

The mean absorbance value comes from two measurements of the water-filled cuvette. The standard uncertainty was estimated during the method validation by twenty repeated measurements.

A_{cal}:
Type A
Method of observation: Direct
Number of observations: 2

No.	Observation
1	0.2992
2	0.3027

Arithmetic Mean: 0.30095 AU
Pooled Standard Deviation: 0.99%
Standard Uncertainty: $2.09 \cdot 10^{-3}$ AU
Pooled Degrees of Freedom: 19

The mean absorbance value comes from the two measurements of the calibrator. The standard uncertainty was estimated during the method validation by twenty repeated measurements.

c_{cal}:
Type B normal distribution
Value: 2.61 mmol/L
Expanded Uncertainty: 0.05 mmol/L
Coverage Factor: 2

The value and expanded uncertainty (2.61 ± 0.05 mmol/L) come from a certificate provided by the manufacturer. They were obtained by calibrating an atomic absorption spectrometric procedure (AAS) with a certified reference material (NIST SRM 909b)[19] whose value is traceable to base units in the SI system.

k_{intra}:
Type B normal distribution
Value: 1.0
Expanded Uncertainty: 1.9%
Coverage Factor: 1

The uncertainty due to intra-individual biological variation is taken from the literature.[18]

k_{matrix}:
Type B rectangular distribution
Value: 1.0
Halfwidth of Limits: 0.1%

The certificate informs that the calibrator was prepared from a lyophilised (freeze-dried) serum pool. The analyst has on some occasions noted a small effect from similar materials and wishes to take this into account in the form of an extra uncertainty contribution.

k_{drift}:
Type B rectangular distribution
Value: 1
Halfwidth of Limits: 1%

The laboratory allows a maximum sensitivity drift between calibrations of ±1%

k_{pre}:
Type B normal distribution
Value: 0 mmol/L
Expanded Uncertainty: 0.018 mmol/L
Coverage Factor: 1

Information from the scientific literature is used. A pre-analytical contribution of 0.7% at physiological concentrations is stated.[17] The uncertainty is calculated as 0.7% of 2.53 mmol/L = 0.144 mmol/L. A term rather than a factor is used since there are many contributions to this component, which can be suspected not to be concentration dependent.

***d*:**
The method covers well the normal range of calcium concentrations so no dilution is needed.

2.4.2. Quantifying uncertainty components in the glucose determination

c_0:
Constant
Value: 0 mmol/L

A blank solution (distilled/deionized water) is used as the second calibrator.

A_s:
Type A
Method of observation: Direct
Number of observations: 1

No.	Observation
1	0.1153

Arithmetic Mean: 0.115300 AU
Pooled Standard Deviation: 0.50%
Standard Uncertainty: $576 \cdot 10^{-6}$ AU
Pooled Degrees of Freedom: 19

This absorbance value comes from the sample while the uncertainty was estimated during the method validation.

A_0:
Type A
Method of observation: Direct
Number of observations: 2

No.	Observation
1	-0.0011
2	-0.0012

Arithmetic Mean: $-1.1500 \cdot 10^{-3}$ AU
Pooled Standard Deviation: 16%
Standard Uncertainty: $127 \cdot 10^{-6}$ AU
Pooled Degrees of Freedom: 19

This absorbance value comes from the water-filled cuvette while the uncertainty was estimated during the method validation.

A_{cal}:
Type A
Method of observation: Direct
Number of observations: 2

No.	Observation
1	0.2679
2	0.2634

Arithmetic Mean: 0.26565 AU
Pooled Standard Deviation: 0.4%
Standard Uncertainty: $889 \cdot 10^{-6}$ AU
Pooled Degrees of Freedom: 19

This absorbance value comes from the calibrator while the uncertainty was estimated during the method validation.

c_{cal}:
Type B normal distribution
Value: 10.50 mmol/L
Expanded Uncertainty: 0.10 mmol/L
Coverage Factor: 2

The value and expanded uncertainty (10.5 ± 0.10 mmol/L) come from a certificate provided by the manufacturer. The values were obtained by applying a reference method (isotope dilution mass spectrometry) together with reference materials (NIST SRM 917a and SRM 965).[19] The value is traceable to base units in the SI system.

k_{intra}:
Type B normal distribution
Value: 1.0
Expanded Uncertainty: 6.5%
Coverage Factor: 1

The uncertainty due to intra-individual biological variation is taken from the literature.[18]

k_{matrix}:
Constant
Value: 1.0

The certificate informs that the calibrator was prepared from a pool of fresh frozen human serum without any additives. There is no reason to suspect a matrix effect.

k_{drift}:
Type B rectangular distribution
Value: 1.0
Halfwidth of Limits: 1%

The laboratory allows a maximum sensitivity drift between calibrations of ± 1%.

k_{pre}:
Type B normal distribution
Value: 0 mmol/L
Expanded Uncertainty: 0.144 mmol/L
Coverage Factor: 1

Information from the scientific literature is used. A pre-analytical contribution of 3.2% at physiological concentrations is stated.[17] The uncertainty is calculated as 3.2% of 4.5 mmol/L = 0.144 mmol/L.

d:
A sample volume, V_1 of 50 µL is manually diluted by an addition of 450 µL of 0.9% NaCl, V_2 outside the instrument. The uncertainties of the volumes (below) come from information provided by the manufacturer of the pipettes and were confirmed in internal validation work.

V_2:
Type B normal distribution
Value: 450 µL
Expanded Uncertainty: 0.6%
Coverage Factor: 1

V_1:
Type B normal distribution
Value: 50 µL
Expanded Uncertainty: 0.6% Coverage Factor: 1

2.4.3. Summary of uncertainty components for calcium and glucose

	Calcium			Glucose	
Quantity	**Value**	**Standard Uncertainty**		**Value**	**Standard Uncertainty**
c_0	0.0 mmol/L			0.0 mmol/L	-
A_s	0.29360 AU	$2.91 \cdot 10^{-3}$ AU		0.115300 AU	$576 \cdot 10^{-6}$ AU
A_0	0.060700 AU	$838 \cdot 10^{-6}$ AU		$-1.1500 \cdot 10^{-3}$ AU	$127 \cdot 10^{-6}$ AU
A_{cal}	0.30095 AU	$2.09 \cdot 10^{-3}$ AU		0.26565 AU	$889 \cdot 10^{-6}$ AU
c_{cal}	2.6100 mmol/L	0.0250 mmol/L		10.5000 mmol/L	0.0250 mmol/L
k_{intra}	1.0000	0.0190		1.0000	0.0650
k_{matrix}	1.000000	$577 \cdot 10^{-6}$		-	-
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$		1.00000	$5.77 \cdot 10^{-3}$
k_{pre}	0.0 mmol/L	0.0180 mmol/L		0.0 mmol/L	0.144 mmol/L
V_2	-	-		450.00 µL	2.70 µL
V_1	-	-		50.000 µL	0.300 µL

2.5. Step 4: Calculating the combined standard uncertainty

In the last step of the evaluation, the value of the measurand and the combined standard uncertainty (u_c) are calculated from the model. There are several ways to calculate the u_c.[3] What we need to do is to propagate the individual uncertainty contributions according to mathematical rules. For simple models this can easily be done manually. Alternatively a spreadsheet (e.g. Excel) can be used.[3] Here we have applied the software GUM Workbench®, which summarises the result of the calculations in a simple and easy to understand table (uncertainty budget).[20]

3. Results and discussion

3.1. Uncertainty budgets for calcium

3.1.1. Case 1: including only uncertainty components in the analytical phase

The table below shows the uncertainty budget for calcium when only uncertainty sources in the analytical phase are considered. The importance of each uncertainty component is given relatively (index in %). As can be seen, the absorbance measurement on the sample is the most important source of uncertainty (43.7%). The allowed drift is a negligible contribution. At the bottom you find the value (2.5302 mmol/L) and combined standard uncertainty (0.0478 mmol/L) for the calcium concentration in the sample.

It is recommended that when results are reported, the uncertainty should be stated as an expanded uncertainty ($U = k \cdot u_c$) with coverage factor k equal to 2. This corresponds approximately to a 95% confidence interval. This is illustrated in the small table below. Similar information for glucose is found in Section 3.2.

Case 1. **Uncertainty budget for c_{Ca} for the analytical phase.**

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.29360 AU	$2.91 \cdot 10^{-3}$ AU	43.6 %
A_0	0.060700 AU	$838 \cdot 10^{-6}$ AU	0.0 %
A_{cal}	0.30095 AU	$2.09 \cdot 10^{-3}$ AU	21.2 %
c_{cal}	2.6100 mmol/L	0.0250 mmol/L	25.7 %
k_{matrix}	1.000000	$577 \cdot 10^{-6}$	0.0 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	9.3 %
c_{Ca}	2.5302 mmol/L	0.0478 mmol/L	

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor
c_{Ca}	2.530 mmol/L	0.096 mmol/L	2.0
F	10.86 (mmol/L)/AU	0.29 (mmol/L)/AU	2.0

3.1.2. Case 2: Pre-analytical and analytical contributions

This evaluation adds the pre-analytical contributions. The uncertainty budget reveals that they do not affect significantly the combined standard uncertainty.

Case 2. **Uncertainty budget for c_{Ca} for the pre-analytical and analytical phases.**

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.29360 AU	$2.91 \cdot 10^{-3}$ AU	38.2 %
A_0	0.060700 AU	$838 \cdot 10^{-6}$ AU	0.0 %
A_{cal}	0.30095 AU	$2.09 \cdot 10^{-3}$ AU	18.6 %
c_{cal}	2.6100 mmol/L	0.0250 mmol/L	22.5 %
k_{matrix}	1.000000	$577 \cdot 10^{-6}$	0.0 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	8.2 %
k_{pre}	0.0 mmol/L	0.0180 mmol/L	12.4 %
c_{Ca}	2.5302 mmol/L	0.0511 mmol/L	

3.1.3. Case 3: including all identified uncertainty components

The table below shows the uncertainty budget for calcium when the also the biological variation is considered. It is obvious that this intra-individual effect has a large influence when it is included in the budget.

Case 3. **Uncertainty budget for c_{Ca} with pre-analytical, analytical and patient-related contributions.**

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.29360 AU	$2.91 \cdot 10^{-3}$ AU	20.3 %
A_0	0.060700 AU	$838 \cdot 10^{-6}$ AU	0.0 %
A_{cal}	0.30095 AU	$2.09 \cdot 10^{-3}$ AU	9.9 %
c_{cal}	2.6100 mmol/L	0.0250 mmol/L	11.9 %
k_{intra}	1.0000	0.0190	47.0 %
k_{matrix}	1.000000	$577 \cdot 10^{-6}$	0.0 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	4.3 %
k_{pre}	0.0 mmol/L	0.0180 mmol/L	6.6 %
c_{Ca}	2.5302 mmol/L	0.0701 mmol/L	

3.2. Uncertainty budgets for glucose

3.2.1. Case 1: including only uncertainty components in the analytical phase

Only uncertainty sources in the analytical phase are considered. In this case the allowed drift constitutes a significant part of the uncertainty (table below). But there are a number of other important components, including the sample dilution.

Case 1. **Uncertainty budget for c_{Gluc} for the analytical phase**.

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.115300 AU	$576 \cdot 10^{-6}$ AU	16.3 %
A_0	$-1.150 \cdot 10^{-3}$ AU	$127 \cdot 10^{-6}$ AU	0.3 %
A_{cal}	0.265650 AU	$889 \cdot 10^{-6}$ AU	7.4 %
c_{cal}	10.5000 mmol/L	0.0500 mmol/L	15.1 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	22.2 %
V_2	450.00	2.70	19.4 %
V_1	50.000	0.300	19.4 %
c_{gluc}	45.829 mmol/L	0.562 mmol/L	

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor
c_{gluc}	45.8 mmol/L	1.1 mmol/L	2.0
d	10.00	0.15	2.0
F	39.36 (mmol/L)/AU	0.46 (mmol/L)/AU	2.0

3.2.2. Case 2: pre-analytical and analytical contributions

This evaluation adds the pre-analytical contributions but the effect on u_c is small.

Case 2. **Uncertainty budget for c_{Gluc} for the pre-analytical and analytical phases.**

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.115300 AU	$576 \cdot 10^{-6}$ AU	15.3 %
A_0	$-1.150 \cdot 10^{-3}$ AU	$127 \cdot 10^{-6}$ AU	0.2 %
A_{cal}	0.265650 AU	$889 \cdot 10^{-6}$ AU	6.9 %
c_{cal}	10.5000 mmol/L	0.0500 mmol/L	14.2 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	20.8 %
k_{pre}	0.0 mmol/L	0.144 mmol/L	6.2 %
V_2	450.00	2.70	18.2 %
V_1	50.000	0.300	18.2 %
c_{gluc}	45.829 mmol/L	0.580 mmol/L	

3.2.3. Case 3: including all identified uncertainty components

To study the influence of the biological variation, we need to change now the situation to reflect more a situation when the clinician may be interested in this information. In the table below we have modelled an example where the glucose concentration is close to the upper limit of the normal range. It is obvious that the intra-individual variation has a large influence when it is included in the budget.

Case 3. **Uncertainty budget for c_{Gluc} with pre-analytical, analytical and patient-related contributions.**

Quantity	Value	Standard Uncertainty	Index
c_0	0.0 mmol/L		
A_s	0.152000 AU	$760 \cdot 10^{-6}$ AU	0.5 %
A_0	$-1.150 \cdot 10^{-3}$ AU	$127 \cdot 10^{-6}$ AU	0.0 %
A_{cal}	0.265650 AU	$889 \cdot 10^{-6}$ AU	0.2 %
c_{cal}	10.5000 mmol/L	0.0500 mmol/L	0.5 %
k_{intra}	1.0000	0.0650	86.4 %
k_{drift}	1.00000	$5.77 \cdot 10^{-3}$	0.7 %
k_{pre}	0.0 mmol/L	0.144 mmol/L	11.7 %
c_{gluc}	6.027 mmol/L	0.421 mmol/L	

3.3. Use of the uncertainty information

Evaluating the measurement quality is a task of the laboratory. IQC procedures, e.g. the control chart, can provide information on the combined effect of several uncertainty components, and assures that the measurements are under statistical control. When using IQC samples, any differences in matrix, stability and homogeneity compared to patient samples may affect performance characteristics (mainly precision) and should be considered in the uncertainty evaluation. Also note that the control chart may not always cover the pre-analytical work.

EQA schemes serve as a check of the evaluated uncertainty but they do not replace the laboratory's own evaluation. Differences of the EQA samples (matrix, stability and homogeneity) compared to patient samples may affect performance characteristics. In addition there are limitations in the statistical evaluation of participants' results. The traceability and uncertainty of values assigned to the test materials must be known when uncertainty is discussed.

3.4. Summary and conclusions

The table below summarises the combined standard uncertainties (absolute and relative) for the three cases.

Combined standard uncertainty (u_c) (absolute, and relative in %) for the three cases. The nominal concentrations are 2.530 mmol/L for calcium and 45.83 (cases 1-2) and 6.027 mmol/L (case 3) for glucose.

Case	Sources of uncertainty	u_c Calcium mmol/L	u_c Glucose mmol/L
1	Analytical	0.048 (1.9%)	0.56 (1.2%)
2	Analytical and pre-analytical	0.051 (2.0%)	0.58 (1.3%)
3	Analytical, pre-analytical and patient-related	0.070 (2.8%)	0.42 (7.0%)

An EQA scheme organiser reports systematic errors between –3% and +0.2%, and a long-term precision between 0.5 and 4% for routine calcium determinations.[21] The differences between laboratories can hence be fairly large. For glucose, another organiser has set target values for the long-term intermediate (within laboratory) precision, and for the systematic error, of 2.1%, and ±6% respectively.[22] The performance in one round (spread between participants) was 4% for photometric methods. The examples in this report may serve as inspiration when the measurement quality is evaluated according to new international guidelines.

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