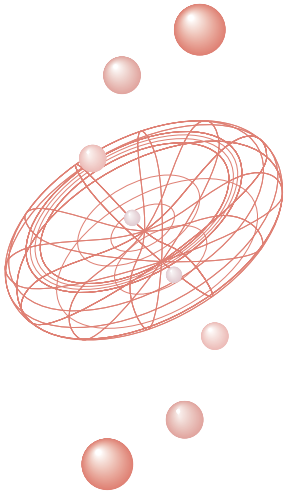
REVIEW ARTICLE



Measurement Uncertainty of Values Assigned to Sysmex Haematology Calibrator SCS-1000

Christina ROOS[*1], Etsuro SHINKAI[*2] and Keiji FUJIMOTO[*2]

[*1] Product Marketing, Sysmex Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany
[*2] Scientific Affairs, Sysmex Corporation, 1-3-2 Murotani, Nishi-ku, Kobe 651-2241, Japan
Correspondence: Dr. Christina ROOS, Product Marketing, Sysmex Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany. E-mail: roos.christina@sysmex-europe.com

Key Words

Uncertainty, Traceability, ISO 15189, GUM, Blood Cell Counting, Haematology, Calibrator

(Sysmex J Int. 18: 31-37, 2008)

Received 6 June, 2008; Accepted 12 June, 2008

ABSTRACT

The laboratory accreditation system based on ISO 15189 requires medical laboratories to ensure traceability of measurement results and estimate their measurement uncertainty. SCS-1000 - the calibrator for Sysmex haematology analysers - is provided with assigned values traceable to international conventional reference measurement procedures and with measurement uncertainty data, thus enabling the laboratory to fulfill the requirements of ISO 15189 in this respect. Following a short introduction on traceability and measurement uncertainty as fundamental concepts for assuring reliability and comparability of measurement results, this article describes the estimation of measurement uncertainty in general and for the values assigned to SCS-1000, using the erythrocyte count as an example.

INTRODUCTION

Traceability and measurement uncertainty are the basis for assuring reliability and comparability of measurement results, and these concepts have been more and more implemented into the field of laboratory medicine during the last years. In 2002 the Joint Committee for Traceability in Laboratory Medicine (JCTLM)[1)] was set up to support world-wide comparability, reliability and equivalence of measurement results in laboratory medicine. In pursuit of this goal JCTLM promotes the concept of traceability of measurement results to the International System of Units (SI) or to other internationally agreed references. National and international regulations - like the EU directive on *in-vitro* diagnostic medical devices (98/79/EC) - require manufacturers to demonstrate the metrological traceability of values assigned to calibrators and control materials intended to establish or verify trueness of measurement results. Since the introduction of the laboratory accreditation system based on ISO 15189[2)] in 2004, medical laboratories are required to ensure traceability of measurement results and estimate their measurement uncertainty.

Metrological traceability is defined as

> "property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty"[3)].

This means that a measurement result can be traced back to a universally recognized and accepted reference by a system of calibrations and comparisons (the traceability chain). With the traceability chain knowledge is provided of the metrological basis of a measurement result and the way the measurement result is connected to this basis.

ISO 17511[4] specifies procedures how to assure traceability for the field of *in-vitro* diagnostic medical devices. Generally, the ideal end-point of a traceability chain is the definition of the relevant SI unit. Where this is not possible, the metrologically highest reference may be an international conventional reference measurement procedure, or an international conventional reference material. In the field of laboratory medicine there are many cases where the traceability chain ends at lower metrological levels such as the manufacturer's selected measurement procedure, or the manufacturer's working calibrator.[*1]

Information on measurement uncertainty is essential to establish traceability of a measurement result: for each step along the traceability chain knowledge of the measurement uncertainty is required.

Measurement uncertainty is defined as

> "non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used"[3].

Measurand is defined as

> "quantity intended to be measured"[3].

A measurement result is often expressed as a single value[*2] but actually it represents a dispersion of values that can reasonably be attributed to the measurand. Measurement uncertainty provides information on this dispersion by defining an interval where the value of the measurand is believed to lie within with a high level of confidence. It summarizes the major factors that influence the measurement result along the traceability chain and thus represents the extent of possible error in the measurement result.
In general use, the term 'uncertainty' relates to the concept of 'doubt'. In contrast to that, knowledge of the measurement uncertainty implies increased confidence in the validity of the result and is required to decide if a result is adequate for its intended purpose.

EVALUATION OF MEASUREMENT UNCERTAINTY

The "Guide to the Expression of Uncertainty in Measurement" (GUM)[6] first published by ISO in 1993, established general rules for evaluating and expressing measurement uncertainty across a broad spectrum of measurements. This elaborate guide is internationally widely recognized and used as basis for evaluating and expressing measurement uncertainty. The following text gives a short, simplified introduction to the concept for evaluating measurement uncertainty and points out some practical aspects - based on the mathematical concept of the GUM and information given in more concise guides (e.g. the EURACHEM / CITAC Guide CG4[7]).

Step 1

The first step in evaluating measurement uncertainty is to specify the measurand including the relationship between the measurand (output quantity) and the parameters upon which it depends (input quantities; see example given below in "Step 4"). This step is important because it is essential to have a clear understanding of what is being measured and of the factors influencing the measurement result.

Step 2

The second step in the process of evaluating uncertainty is identifying all sources of uncertainty. All relevant sources of uncertainty which influence the measurement result along all steps of the traceability chain have to be taken into account. This includes factors that influence the input quantities specified in the first step. Measurement uncertainty comprises not only the variation of the measurement step itself but also uncertainties of volumes of measuring devices, influence of environmental conditions, variation of sample material, variation in sample preparation and others.

Step 3

The third step is to quantify the contributions from the different sources of uncertainty and to decide which are the relevant contributions. It is important to keep in mind that the value obtained for the overall uncertainty from different sources is almost entirely controlled by the major contributions. In practice a good estimate of measurement uncertainty can be made by identifying and quantifying the most significant sources of uncertainty - thus keeping the effort for evaluating measurement uncertainty within reasonable limits. Methods for the evaluation of uncertainty components are grouped into two categories:

Type A: Methods of evaluation of uncertainty by statistical analysis of series of observations.
Type B: Methods of evaluation of uncertainty by means other than Type A.

In most cases evaluation of the overall uncertainty includes Type A and Type B evaluations. An example for Type A evaluation of an uncertainty component is the calculation of the standard deviation for a series of measurements. Type B evaluations are usually based on scientific judgement using all relevant information available, e.g. manufacturer's specifications, data from calibration certificates, previous measurements. It also includes judgement based on experience with, or general knowledge of, the behaviour of an instrument or the properties of a material. Components evaluated by Type B methods can also be characterised by standard deviations, calculated from assumed probability density func-

Note: [*1] Shinkai et al.[5] give more details of the different types of traceability chains according to ISO 17511[4] and traceability in blood cell counting.
[*2] The term "value" is used instead of "quantity value" for readability.

tions based on the above mentioned information (an example will be given below).
In many cases uncertainty components can be evaluated using available data from method validation or regular quality control measurements - thus the additional experimental work needed to evaluate uncertainty can be reduced.

Step 4

The fourth step is to combine the uncertainties from the different sources. All uncertainty contributions are expressed as standard deviations and these 'standard uncertainties $u(x_i)$' are weighted according to their effect on the result with the sensitivity coefficient c_i. This requires the knowledge of the relation f between the result y (output quantity) and the independent parameters x_i on which it depends (input quantities) - as specified in the first step. These weighted standard uncertainties are combined according to the law of 'propagation of uncertainty' (or 'propagation of error') to give the 'combined standard uncertainty $u_c(y)$'.

$$y = f(x_1, x_2, \ldots x_n)$$

y: result
x_i: independent parameter on which the result y depends
f: relation between the result y and the parameters x_i

$$c_i = \frac{\partial y}{\partial x_i}$$

c_i: influence of x_i on y (sensitivity coefficient, given as partial derivative)

$$u_c(y) = \sqrt{\sum_{i=1}^{n} c_i^2 \cdot u(x_i)^2}$$

$u(x_i)$: standard uncertainty of parameter x_i
$u_c(y)$: combined standard uncertainty of the result y

This formula is only valid if the parameters x_i are independent of each other. If not, covariances have to be taken into account and the relation between $u_c(y)$ and $u(x_i)$ is more complex.

This general formula for $u_c(y)$ can be applied for all relations f between the result y and the parameters x_i. In many cases the result y is calculated only by products and quotients of the parameters x_i and then the general formula can be simplified as follows: The relative combined standard uncertainty (this means the combined standard uncertainty $u_c(y)$ divided by the quantity y) can be calculated from the relative standard uncertainties of the parameters x_i (this means the standard uncertainties $u(x_i)$ divided by the quantities x_i). This is illustrated for a dilution process as an example. Solution A with the concentration C_A is diluted using a microdispenser and a flask with the volumes V_M and V_F. The concentration C_B of the diluted solution B is calculated as follows (in other words: the relation between the output quantity C_B and the input quantities C_A, V_M and V_F is the following):

$$C_B = C_A \cdot \frac{V_M}{V_F}$$

The relative standard uncertainties of the concentration C_A and the volumes V_M and V_F are combined to give the relative combined standard uncertainty of the concentration C_B as follows (this formula can be derived from the general formula by inserting the partial derivatives and dividing the whole equation by C_B):

$$\frac{u_c(C_B)}{C_B} = \sqrt{\left(\frac{u(C_A)}{C_A}\right)^2 + \left(\frac{u(V_M)}{V_M}\right)^2 + \left(\frac{u(V_F)}{V_F}\right)^2}$$

$$\Leftrightarrow u_c(C_B) = C_B \cdot \sqrt{\left(\frac{u(C_A)}{C_A}\right)^2 + \left(\frac{u(V_M)}{V_M}\right)^2 + \left(\frac{u(V_F)}{V_F}\right)^2}$$

Step 5

The final step is to multiply the combined standard uncertainty u_c with a coverage factor k to obtain the expanded uncertainty U.

$$U = k \cdot u_c(y)$$

The expanded uncertainty defines an interval around the measurement result (coverage interval) within which the value of the measurand is believed to lie with a higher level of confidence. In other words, the expanded uncertainty is required to provide an interval which may be expected to encompass a large fraction of the distribution of values which could reasonably be attributed to the measurand[7]. The choice of the coverage factor k depends on the confidence level required, the underlying probability distributions and the number of values used to estimate random effects. For most purposes the coverage factor k = 2 is used. Provided the underlying distributions are normal distributions and the number of values used for estimation of random effects are sufficiently high this corresponds to a confidence level of approx. 95%.

Generally the measurement result y and the expanded uncertainty U are reported as "y ± U [unit]"[*3]. When reporting expanded uncertainty the coverage factor k has to be stated so that the combined standard uncertainty can be recovered for further calculations.
The uncertainty estimate evaluated for a given method in a particular laboratory may be reliably applied to subse-

Note: [*3] There may be cases where the coverage interval is not centred on the measurement result. When the upper and lower value of uncertainty are significantly different they should be evaluated and reported separately.

quent results as long as this is justified by the relevant quality control data and as long as the procedure itself or the equipment is not changed.

SYSMEX CALIBRATOR SYSTEM SCS-1000

The SCS-1000 is designed for calibration resp. verification of calibration of Sysmex haematology analysers and contains stabilized human erythrocytes, fixed mammalian leukocytes, and a platelet component in a medium containing preservatives.

The assigned values for SCS-1000 are determined on Sysmex haematology analysers in the Sysmex quality control laboratories (hereinafter "standard analysers"). The standard analysers are calibrated with fresh human blood samples from healthy donors against the ICSH and CLSI[*4] reference measurement procedures. Thus the SCS-1000 assigned values are traceable to international conventional reference measurement procedures as highest metrological level (according to ISO 17511:2003, section 5.4[4)]). The same applies to results of patient samples measured on Sysmex haematology analysers - located e.g. in medical laboratories - which are calibrated with SCS-1000. As an example, the traceability chain for erythrocytes and leukocytes is shown in ***Fig. 1***.

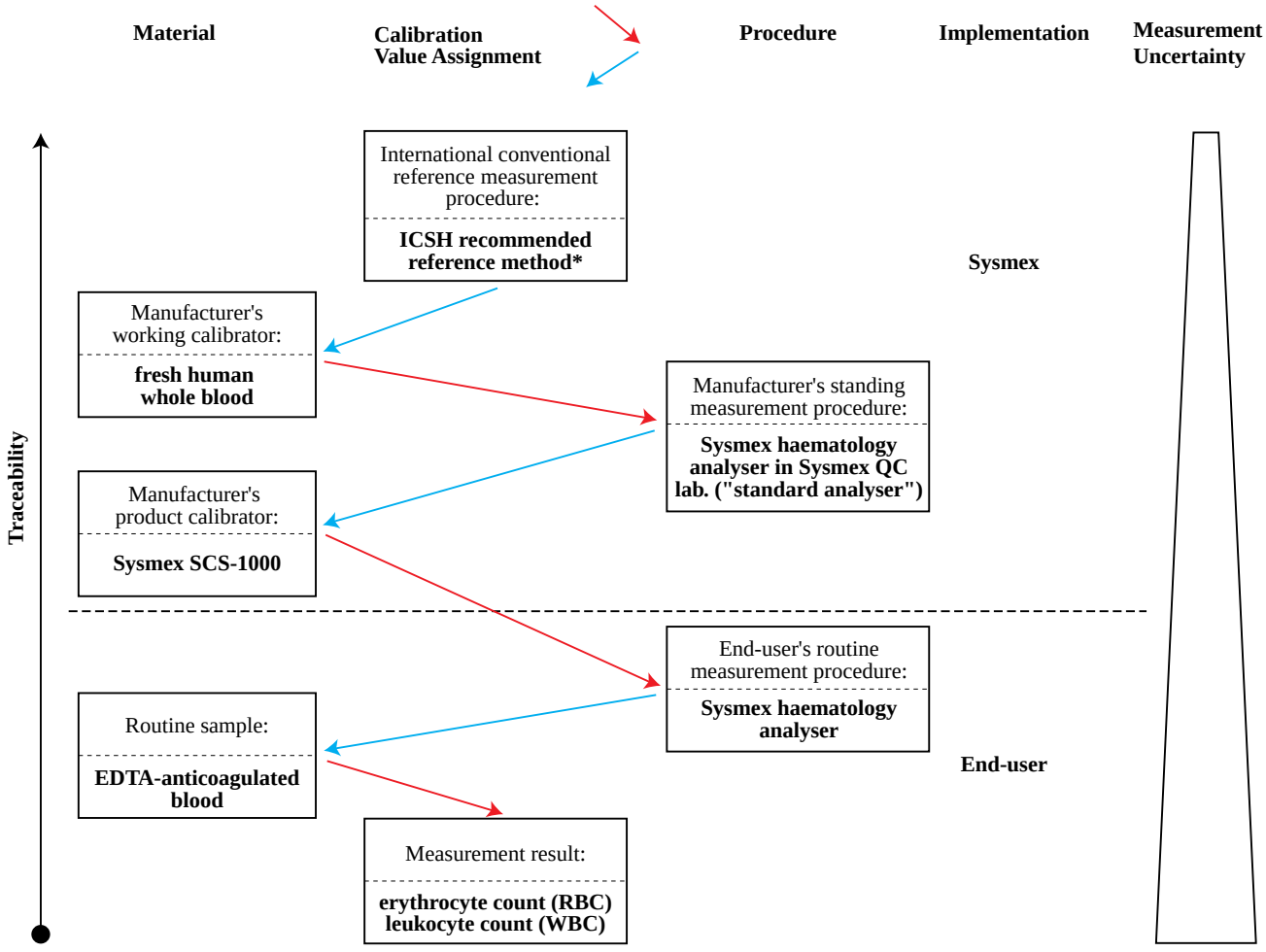


Fig. 1 *Traceability chain of erythrocytes and leukocytes (according to ISO 17511:2003, section 5.4[4)])*

**ICSH Expert Panel on Cytometry: Reference method for the enumeration of erythrocytes and leukocytes. Clin Lab Haematol. 1994; 16: 131-138*

Note: [*4] ICSH: International Council for Standardization in Haematology, CLSI: Clinical and Laboratory Standards Institute

EVALUATION OF MEASUREMENT UNCERTAINTY OF VALUES ASSIGNED TO SCS-1000

For evaluating the measurement uncertainty of values assigned to SCS-1000 all steps along the traceability chain and product properties were taken into account. In the following text the evaluation will be described in detail for erythrocytes (red blood cells, RBC) as an example. The same principles apply to leukocytes, haemoglobin, haematocrit and platelets.

The first step along the traceability chain (see ***Fig. 1***) is the determination of the RBC count for fresh human blood samples with the reference measurement procedure. For this determination the blood samples are diluted using a volumetric flask and a positive-displacement type microdispenser which is calibrated by weighing the dispensed volume of water. The concentration of RBC in the diluted sample is measured with a single-channel semiautomated blood cell counter (SCC) using the impedance method. A certain volume of diluted sample is aspirated through the aperture of the detection unit driven by a mercury-filled manometer. The count obtained is corrected for coincident passage of RBC through the aperture and multiplied by the sample dilution to obtain the RBC count of the blood sample.

Fig. 2 shows the sources of uncertainty for the determination of the RBC count with the reference measurement procedure. The evaluation of the uncertainty components is done by Type A and Type B methods. An example for Type A evaluation is the quantification of the uncertainty caused by variation in repeated measurements of the diluted blood sample - the standard deviation of the count results is calculated and then converted into the standard deviation of the mean count result as follows:

$$s_{Mean} = \frac{s}{\sqrt{n}}$$

s: standard deviation of the count results
n: number of count results
s_{Mean}: standard deviation of mean count result

The quantification of the uncertainty of the volume of the flask used for diluting the blood sample is an example for Type B evaluation. This is done by using the information given by the manufacturer. The volume of the flask V_F is given as 1000 ± 0.4 mL without further information about the underlying distribution. Therefore a rectangular distribution is assumed (i.e. the volume can take all values between 999.6 and 1000.4 mL with the same probability) and the width a = 0.4 mL is transformed into a standard uncertainty according to general statistical procedures using the following formula[7]:

$$u(x) = \frac{a}{\sqrt{3}} \quad \rightarrow \quad u(V_F) = \frac{0.4\ mL}{\sqrt{3}} = 0.23\ mL$$

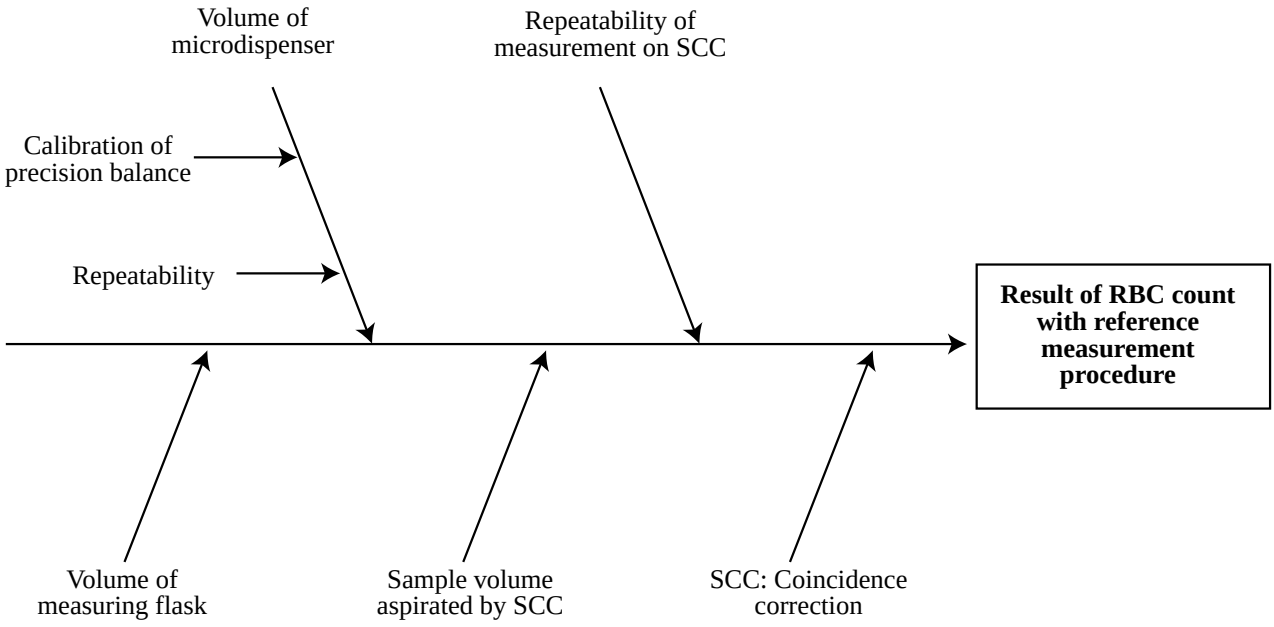


Fig. 2 *Determination of the RBC count for human blood samples with the reference measurement procedure: Identifying sources of uncertainty*

All standard uncertainties were combined according to the law of propagation of uncertainty to give the combined standard uncertainty of the reference measurement procedure results for blood samples.
The next step along the traceability chain is measuring the blood samples on the Sysmex haematology analysers located in the Sysmex quality control laboratories (standard analysers) and - if necessary - changing the calibration of the standard analysers to meet the reference measurement procedure results. The uncertainty connected with this step is caused by the variation of repeated measurements on the standard analysers. It is expressed as the standard deviation of the measurement results and converted into the standard deviation of the mean result as described above. Combining this standard uncertainty with the combined standard uncertainty of the reference measurement procedure results gives the combined standard uncertainty of the calibration of the standard analysers.
Finally the assigned values for the SCS-1000 are determined by measurement on the standard analysers which are calibrated against the reference measurement procedure. As before, the uncertainty of this measurement step is expressed as the standard deviation of the measurement results and converted into the standard deviation of the mean result.

The sources for uncertainty of values assigned to SCS-1000 are summarized in ***Fig. 3***. For evaluating the uncertainty of the assigned values not only the uncertainty contributions from all above mentioned steps were taken into account but also uncertainties connected with product properties were investigated. Uncertainty connected with limitations of the stability of SCS-1000 during shelf life were quantified using data from multiple lots and included in the overall uncertainty of the assigned values. Vial-to-vial variation was evaluated for multiple lots by "Analysis of variances (ANOVA)". The results showed that there is no significant vial-to-vial variation for SCS-1000.

The combined standard uncertainty of values assigned to SCS-1000 is calculated by combining the uncertainties of
1) the results for the human blood samples with the reference measurement procedure,
2) the variation of the measurement of the human blood samples on the standard analysers,
3) the variation of the measurement of SCS-1000 on the standard analysers and
4) the limitations of the stability of SCS-1000 during shelf life.

The expanded uncertainty was calculated using a coverage factor k = 2.

All uncertainty contributions were quantified using data from several calibration measurements and multiple lots of SCS-1000. Thus, quantification of random effects is based on a high number of observations. The results for the uncertainty of the assigned values (given as relative uncertainties) are valid for all lots of SCS-1000 - as long as there are no changes of the procedures applied which would make re-evaluation necessary.

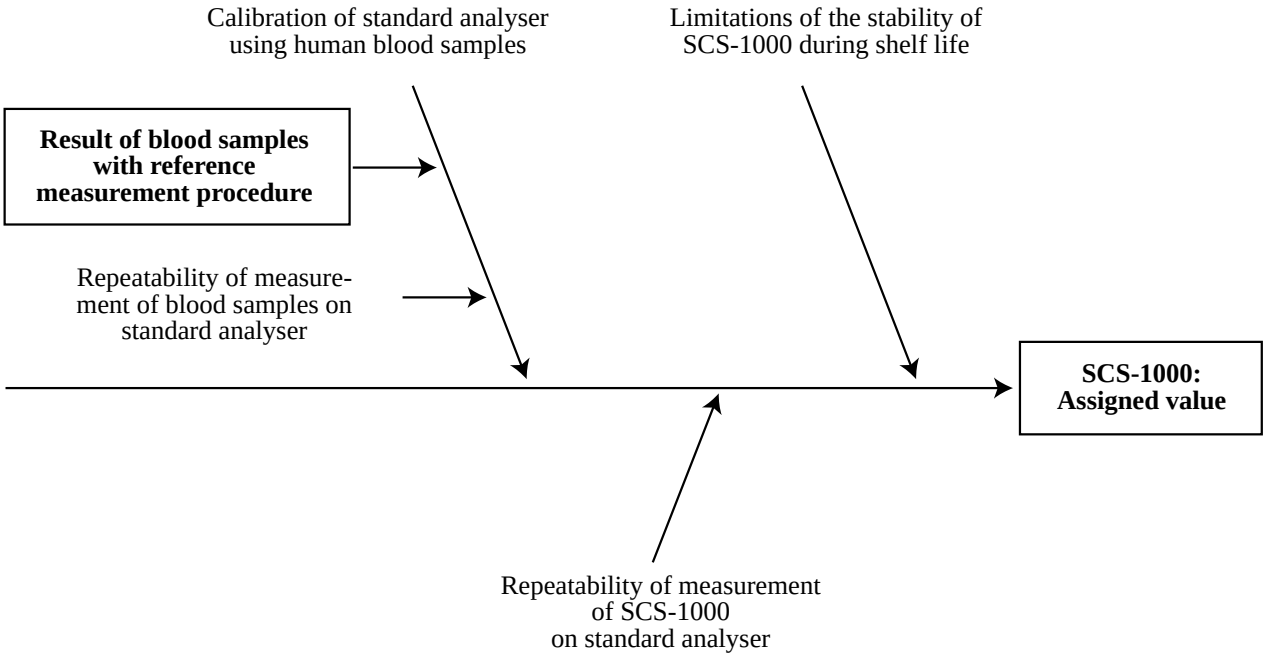


Fig. 3 *Identifying sources of uncertainty of values assigned to SCS-1000*

CONCLUSION

With SCS-1000 a calibrator product for Sysmex haematology analysers is provided with assigned values traceable to international conventional reference measurement procedures (according to ISO 17511:2003, section 5.4[4]) and with measurement uncertainty data determined according to the mathematical concept of the GUM[6].[*5] Use of SCS-1000 enables the laboratory to ensure the traceability and calculate the measurement uncertainty of their patient sample results and thus fulfill the respective requirements of ISO 15189[2].

The uncertainty of the values assigned to SCS-1000 is one component in the calculation of the combined uncertainty of the calibration of the laboratory's analyser. (It is important to keep in mind that the values given on the SCS-1000 uncertainty data sheet are expanded uncertainties. Before using them in further calculations they have to be converted back to standard uncertainties by dividing them by the coverage factor k = 2.) For evaluating the overall uncertainty budget for the patient sample results all relevant factors influencing the results should be taken into account. These may include sampling, sample preparation, sample portion selection, calibrators, reference materials, input quantities, equipment used, environmental conditions, condition of the sample and changes of operator[2]. As mentioned above it is important to keep in mind that the overall uncertainty is controlled by the major contributions and that it is sufficient to concentrate on the most significant sources of uncertainty. When information on uncertainty is given it should be clear what the uncertainty pertains to and which parts of the entire testing process (e.g. sampling, sample preparation, the measurement step itself) were included in the calculation.

This paper is focusing on measurement uncertainty. But the measurement step itself is only one part of the entire testing process, and often the uncertainty connected with the measurement step is small compared to uncertainty arising from other sources. Many errors may occur in the preanalytical and postanalytical phase and careful attention paid to such aspects is an important part of the efforts undertaken to report reliable measurement results.

References

1) *Joint Committee for Traceability in Laboratory Medicine (JCTLM). (online), http://www.bipm.org/en/committees/jc/jctlm/, (accessed 2008-06-05).*
2) *ISO 15189: Medical laboratories - Particular requirements for quality and competence. 2007. 40p.*
3) *ISO/IEC Guide 99: International vocabulary of metrology - Basic and general concepts and associated terms (VIM). 3rd ed. Geneva: 2007. 92p.*
4) *ISO 17511: In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials. 2003. 23p.*
5) *Shinkai E, Shirakami A, Fujimoto K. Traceability and Uncertainty in Blood Cell Counting. Sysmex J Int. 2008; 18(1): 1-11.*
6) *ISO/IEC Guide 98: Guide to the Expression of Uncertainty in Measurement (GUM), Geneva: 1995. 101p.*
7) *EURACHEM/CITAC Guide CG 4 Quantifying Uncertainty in Analytical Measurement. 2nd ed. 2000. 120p.*

Note: [*5] Uncertainty data sheets are issued for the different Sysmex haematology analysers and can be requested from your local Sysmex representative. In Japan uncertainty data are supplied as part of the calibration service (for a description of the procedures applied in Japan please refer to Shinkai et al.[5]) .