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SEED HAEMATOLOGY

Introduction to the concepts of metrological traceability and measurement uncertainty and their application for Sysmex's haematology calibrators

This article gives a short introduction to the concepts of metrological traceability and measurement uncertainty (hereinafter 'traceability' and 'uncertainty') for readers who are not familiar with them. It also includes a short description of the steps needed to evaluate uncertainty according to the 'Guide to the Expression of Uncertainty in Measurement (GUM)' [1]. The article further explains how traceability is assured for the Sysmex haematology calibrators XN CAL, XN CAL PF and SCS-1000 and describes how the uncertainty of these calibrators was evaluated.

Additionally, this article shortly explains how to use the uncertainty determined for the calibrators in evaluating the uncertainty of results of patient samples measured on Sysmex haematology systems. More information on the evaluation of the uncertainty of results of patient samples will be available later.

Introduction

Traceability and measurement uncertainty are important concepts not only in the analytical laboratory but also in everyday life – although we are generally not aware of this.

For example, if we buy 200 g of cheese in the supermarket and trust that we have actually received 200 g, we are assuming that the scales in the supermarket are correctly calibrated and that their results are traceable to a recognised standard. On the other hand, if we suspected the supermarket's scales of being incorrect, we would weigh the cheese at home. Suppose we measured 202 g, then we would intuitively decide that this represents acceptable agreement between the results of the two sets of scales – without thinking about the concept of uncertainty.

Traceability and uncertainty are the basis for assuring reliability and comparability of measurement results. They are indispensable for answering questions such as:

- 'Are these two results from different laboratories in agreement?'
- 'Is the white blood cell concentration below the limit value?'
- 'Does this result show an increase in haemoglobin concentration compared with the value obtained on the previous day?'

What does traceability mean?

In the case of the scales in the supermarket, traceability could be described as follows: The scales are checked regularly by the verification authority and, if necessary, calibrated (or in this case 'verified'), which is confirmed by the verification seal on the scales. The check is conducted by a measurement of certified weights (mass standards) of high accuracy, the exact masses of which are determined by the competent national institute. The masses are determined by comparison measurements against the national standard, the mass of which in turn was determined by comparison measurements against the international standard. This 'international kilogram prototype' – better known as the 'original kilogram' – is housed at the 'International Bureau of Weights and Measures' (BIPM) near Paris, and the mass of the original kilogram forms the definition of the unit known as 'kilogram'.

This simplified desription (Fig. 1) skips a few steps – but it still illustrates how the result of the scales in the supermarket is traceable to the original kilogram as the internationally recognised reference.

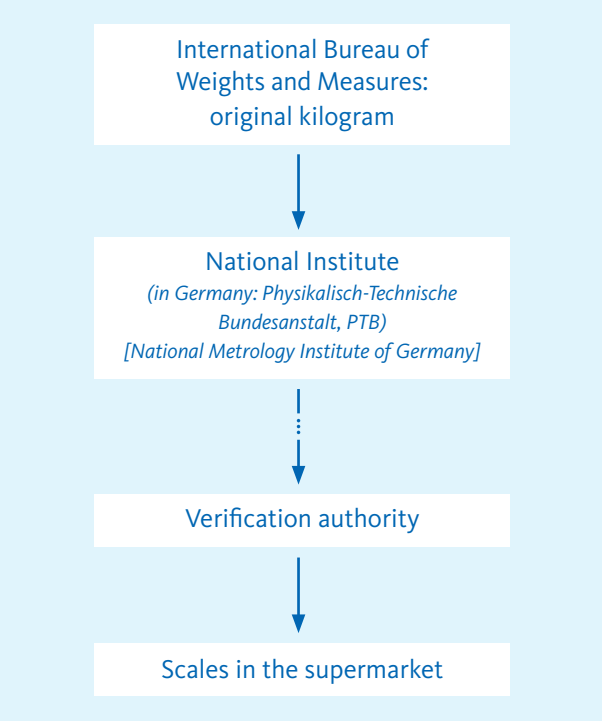

Fig. 1 *Traceability chain for supermarket scales*

The 'International vocabulary of metrology (VIM)' [2] defines 'metrological traceability' 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.'

The 'unbroken chain of calibrations' is known as 'traceability chain'. It is by this chain that the result is related to a generally recognised and accepted reference. In our example, the original kilogram is the reference to which the measurement result of the scales in the supermarket is traced. The reference may be a material or a measurement method.

Every step in the traceability chain is associated with uncertainties, which must be taken into consideration when evaluating the overall uncertainty of the result. Statements about uncertainty are an important part of traceability. This is easy to understand by asking oneself how much trust could be placed in scales giving a result of 200 g that is indeed traceable to the original kilogram, but with an uncertainty of ± 50 g – this would be much too inaccurate for scales in the supermarket.

What does measurement uncertainty mean?

A measurement result is often expressed as a single value but it actually represents a distribution of values that can be reasonably attributed to the measurand. The reason is that no measurement is absolutely exact because the outcome of a measurement is influenced by diverse factors. This means that the measurement result can only be an estimate of the ('unknowable') true value* of the measurand. The uncertainty defines an interval around the measurement result that is expected to encompass a large fraction of this distribution of values – in other words, an interval within which the true value of the measurand lies with high probability. Another way of interpretation is to understand uncertainty as a measure of the possible error in the measurement result. Whichever way, the uncertainty is a quantitative indication of the quality of the result.

$100.5\,g \longrightarrow 100.5 \pm 0.3\,g\ (k=2)$

(The coverage factor k will be explained below.)

* The concept of 'true value' is subject to an ongoing debate. For example, the GUM omits 'true' as redundant and just uses the term 'value of the measurand'. Nevertheless, the term 'true value of the measurand' is used here to facilitate understanding.

Let us consider the uncertainty of pipetting, as an example. The pipetted volume is influenced by the reproducibility of the pipetting operation and by the calibration of the pipette. In our example, this calibration is achieved by repeated pipetting and weighing of water under controlled conditions. This calibration of the pipette is influenced by the reproducibility of the pipetting operation during calibration, by the density of the water and by the calibration of the balance. The pipetted volume depends on all of these influencing factors, and the uncertainty of the pipetted volume sums up all of the individual errors from these influences (sources of uncertainty).

The 'International vocabulary of metrology (VIM)' [2] defines 'measurement uncertainty' as

> 'Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.'

Why are traceability and measurement uncertainty important?

By means of traceability to a generally recognised reference, such as the original kilogram or an internationally recognised reference method, measurement results are placed on a generally recognised basis to serve as a foundation for the trueness of the results – an important building block for the reliability of results. The connection to a common basis is an important prerequisite for the comparability of results. The traceability chain shows how the result is linked to this basis and therefore provides important information about the steps and materials with which traceability is assured.

The uncertainty defines the interval within which the true value of the measurand lies with a stated, high probability. This information is essential to be able to meaningfully compare results with one another or with a given value/value range (such as a limit value or a reference range). The uncertainty indicates the degree of agreement to be expected between different results. It is an important tool for evaluating whether a procedure yields sufficiently reliable results to meet clinical requirements or whether there is need for improvement.

In the latter case, knowledge of the sources of uncertainty and their relative magnitude helps to identify opportunities for modifying the procedure to improve the quality of results.

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 a measurement result.

How is the traceability of measurement results assured in laboratory medicine?

In most quantitative measurement procedures used in laboratory medicine, calibration is performed with calibrators provided by the manufacturer. According to European Union Directive 98/79/EC on in-vitro diagnostic medical devices ('IVD Directive'), the manufacturer has to assure metrological traceability of values assigned to calibrators to references of higher metrological order. International Standard ISO 17511 [3] specifies procedures for assuring traceability depending on the nature of the reference of highest metrological order.

In the above-illustrated example of the scales in the supermarket, the measurement result can be traced to the definition of the unit 'kilogram' (i.e. the original kilogram) as the reference of highest metrological order – the ideal end point of a traceability chain.

In the field of laboratory medicine, however, only few measurement procedures can be traced to the definition of fundamental physical units. In many cases internationally recognised reference materials or methods constitute the end point of the traceability chain – in such cases the reference of highest metrological order is established by international agreements, i.e. recommendations of international scientific organisations or the World Health Organisation (WHO). On the other hand, there are also many cases in which no internationally recognised reference materials or methods are available but instead a selected measurement procedure of the manufacturer of a test constitutes the end point of the traceability chain.

Traceability for Sysmex haematology systems

The Sysmex haematology calibrators XN CAL, XN CAL PF and SCS-1000 are designed for the calibration of Sysmex haematology systems. The assigned values of XN CAL, XN CAL PF and SCS-1000 are traceable to internationally recognised reference methods for WBC, RBC, HGB, HCT and PLT, according to the recommendations of International Council for Standardization in Haematology (ICSH) and Clinical and Laboratory Standards Institute (CLSI). For RET% the manual eye count (as described by CLSI) is used.*

***Note:** *The method used for RET% (manual eye count) is, strictly speaking, no reference method because of its inherent inaccuracy. Nevertheless, this method is used as comparison method (in place of a reference method) because currently there is no method available for quantifying reticulocytes that has a sufficiently high accuracy to be recognised as a reference method.*

The traceability chain is based on Section 5.4 of ISO 17511 [3] and is illustrated in Fig. 2. In contrast to the simplified form in Fig. 1, the materials used are shown on the left and the measurement procedures on the right.

The Sysmex QC laboratories house serial models of every type of Sysmex haematology system (hereinafter: 'Sysmex haematology reference analyser' or shorter 'Sysmex reference analyser') that are used to determine the assigned values of Sysmex calibrators and thus serve as reference for the users' Sysmex haematology systems in the market. The traceability chain is established as follows: The Sysmex reference analysers are regularly calibrated with fresh human blood samples directly against the reference methods recommended by ICSH and CLSI. This means that the fresh blood samples are measured in the QC laboratories using the reference methods and the Sysmex reference analysers. If necessary, the calibration of the Sysmex reference analysers is adjusted to make the results agree with those determined according to the reference methods. The assigned values for the Sysmex calibrators XN CAL, XN CAL PF and SCS-1000 are determined on the Sysmex reference analysers. The Sysmex calibrator is measured on the user's Sysmex haematology system, and the calibration of the system is adjusted, if necessary. Through this chain the results of patient samples measured on this user's system are traceable to the internationally recognised reference methods.

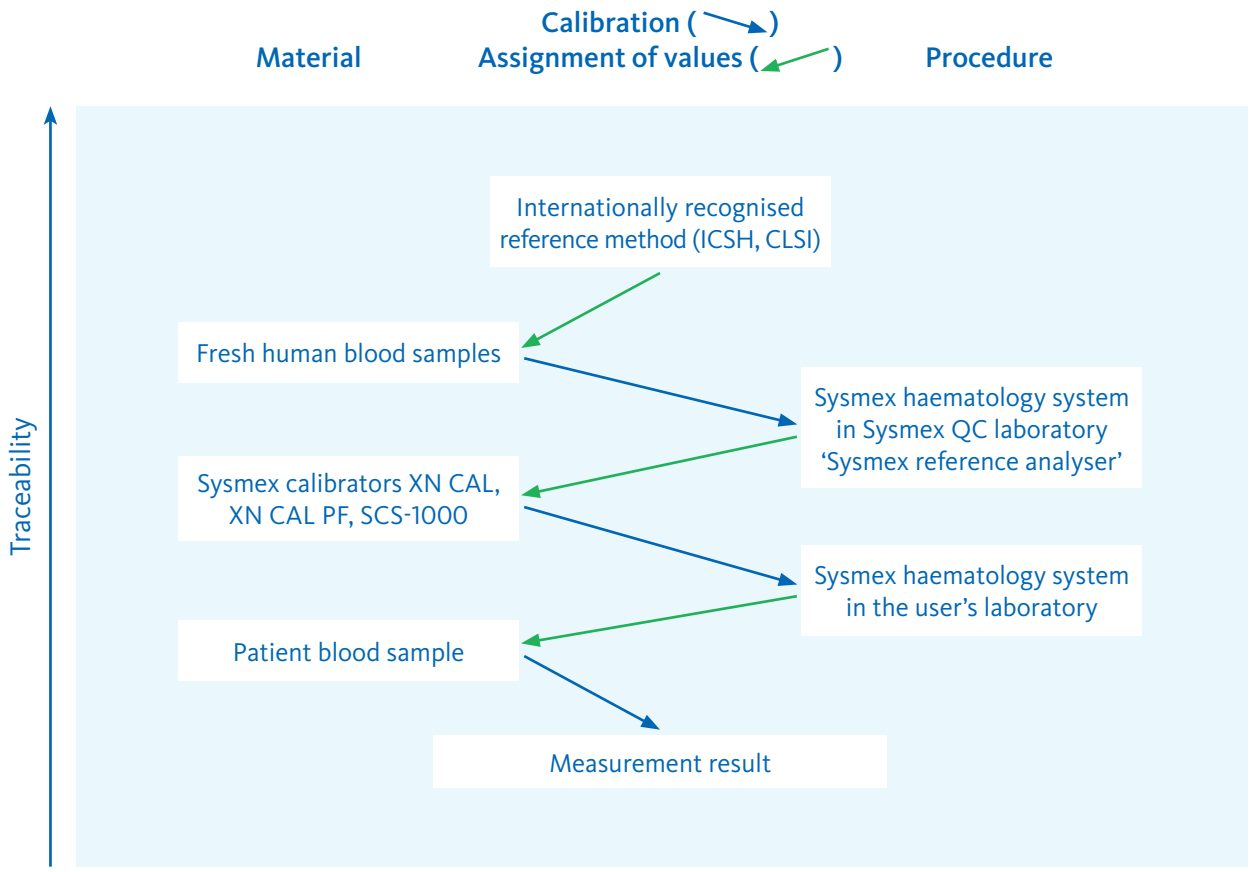

Fig. 2 *Traceability chain according to Section 5.4 of ISO 17511 [3]*

How is measurement uncertainty evaluated and what does 'expanded uncertainty' mean?

An evaluation of measurement uncertainty may follow different approaches, since there is no 'one and only' method how to do it. The main reference for the evaluation of uncertainty is the 'Guide to the Expression of Uncertainty in Measurement (GUM)' [1]. ISO 17511 [3] recommends the principles given in the GUM to be followed for evaluating the uncertainty of calibrators. The evaluation of the uncertainty of Sysmex haematology calibrators is based on these principles.

The general steps of the GUM concept are described in the following paragraphs. To understand them provides valuable knowledge of the subject, which will be helpful even in cases where the evaluation of uncertainty is not exactly following these steps.

The first step in evaluating the uncertainty is to clarify on which items the result depends – including the entire traceability chain – and their mathematical relation.

The second step is identifying all relevant sources of uncertainty. This includes factors that influence the result itself or the items on which the result depends. For this purpose all steps of the traceability chain and the entire course of the sample should be considered. In order to keep the time and effort for the evaluation of the uncertainty within reasonable limits, it should be kept in mind that the overall uncertainty is almost entirely controlled by the major contributions, whereas small contributions have virtually no influence. This means that the evaluation of the uncertainty can be restricted to the most important influencing factors. In the above-mentioned example of the pipette the water temperature is measured and the density calculated accordingly. In doing so, the uncertainty associated with determining the density of the water is negligibly small and does not have to be considered in calculating the overall uncertainty of the pipetted volume.

The third step is to quantify the contributions to the uncertainty. As an example, this can be done by statistical analysis of experimental data (Type A evaluation) or by using information, such as a manufacturer's specifications, data of calibration certificates, estimates based on experience with or expert knowledge of the materials and instruments, or others (Type B evaluation). In many cases it will be possible to draw on data from method validation or quality control.

According to the mathematical concept of the GUM, all contributions are expressed in the form of standard deviations ('standard uncertainty') and in a fourth step are combined according to the law of propagation of errors to obtain the 'combined standard uncertainty'.

The final step is to calculate the 'expanded uncertainty' by multiplying with a coverage factor k. The expanded uncertainty defines an interval within which the true value of the measurand is believed to lie with high probability. In most cases a coverage factor of $k = 2$ is used; this corresponds to a probability (or 'confidence level') of approximately 95% (provided certain statistical conditions are satisfied). When reporting uncertainty, the coverage factor always has to be stated as well: on the one hand so that the underlying probability can be identified and on the other hand so that the non-expanded uncertainty (i.e. the uncertainty in the form of a simple standard deviation) can be calculated, if this is needed for further calculations. This is the case, for example, when the uncertainty of the assigned value of a calibrator is used as a contribution in calculating the overall uncertainty for the results of patient samples.

The modelling method described in the GUM (based on a known mathematical relationship between the result and the items the result depends upon) is not easily applied in medical laboratories due to various reasons. One of them is that measuring systems – like haematology analysers – are often 'closed' systems and not open to a statistical evaluation of the individual sources of uncertainty. Nevertheless, the general steps can be followed: identifying the relevant sources of uncertainty, quantifying and combining the major contributions to uncertainty, and finally calculating the expanded uncertainty. Using data from internal quality control, which summarize several sources of uncertainty, as one part of the overall uncertainty of results of patient samples is a useful approach in medical analysis.

Evaluation of the measurement uncertainty of Sysmex calibrators

The uncertainties of the assigned values of the Sysmex calibrators XN CAL, XN CAL PF and SCS-1000 were evaluated according to the GUM concept. Contributions to uncertainty from various sources along the whole traceability chain were taken into account, including

- reference methods, and pipettes used during these procedures,
- measurements performed to calibrate the Sysmex haematology reference analysers against the reference methods,
- measurements performed to determine the assigned values on the Sysmex haematology reference analysers, and
- properties of the calibrators (vial-to-vial and lot-to-lot variation, stability over time, and so on).

The expanded uncertainty was calculated by multiplying the combined standard uncertainty with a coverage factor of $k = 2$. This defines an interval with a confidence level of approximately 95%.

Since data were collected from many calibrations and many different lot numbers, the data stated for the uncertainty of assigned values are valid for all lots of the specified Sysmex calibrator.

How can you use the uncertainty of Sysmex calibrators to evaluate the uncertainty of the results of your patient samples?

To evaluate the measurement uncertainty of results of patient samples, all relevant components associated with the actual measurement process have to be considered. For Sysmex haematology systems and parameters that are calibrated with XN CAL, XN CAL PF or SCS-1000, this includes

1. the uncertainty of the calibration of the user's system, and
2. factors that influence the precision of the measurement and the stability of the user's system over time.

The first part, the uncertainty of the calibration, can be calculated by combining the standard uncertainty of the assigned values of the calibrator (% values, obtained by dividing the expanded uncertainty by 2) with the imprecision of the measurement of the calibrator on the user's system. To calculate this imprecision the CV% of the measurements has to be divided by the square root of the number of measurements. This is done to obtain the CV% of the mean.

The factors influencing the measurement precision and system's stability include variations in the handling of the sample, fluctuations of the system over time due to electromechanical fluctuations, changes of operator, reagent exchanges, user maintenance, variation of environmental conditions, etc. These factors can be captured by the intermediate precision from the internal quality control (IQC) measurements (expressed as coefficient of variation, CV%).

Combining these two components – the combined standard uncertainty of the calibration and the CV% from the IQC – results in a *combined standard uncertainty for the results of patient samples,* which includes not only the uncertainty connected with the actual measurement on the user's system but also the uncertainty components along the whole traceability chain (see Fig. 3). While more and more uncertainty components add up over the traceability chain, the measurement uncertainty increases with each step and is highest for the result of a patient sample, which includes all uncertainty components.

Again, as the last step the *expanded uncertainty of the results of patient samples* is calculated by multiplying the combined standard uncertainty (that has been obtained as described above) with a coverage factor of $k = 2$.

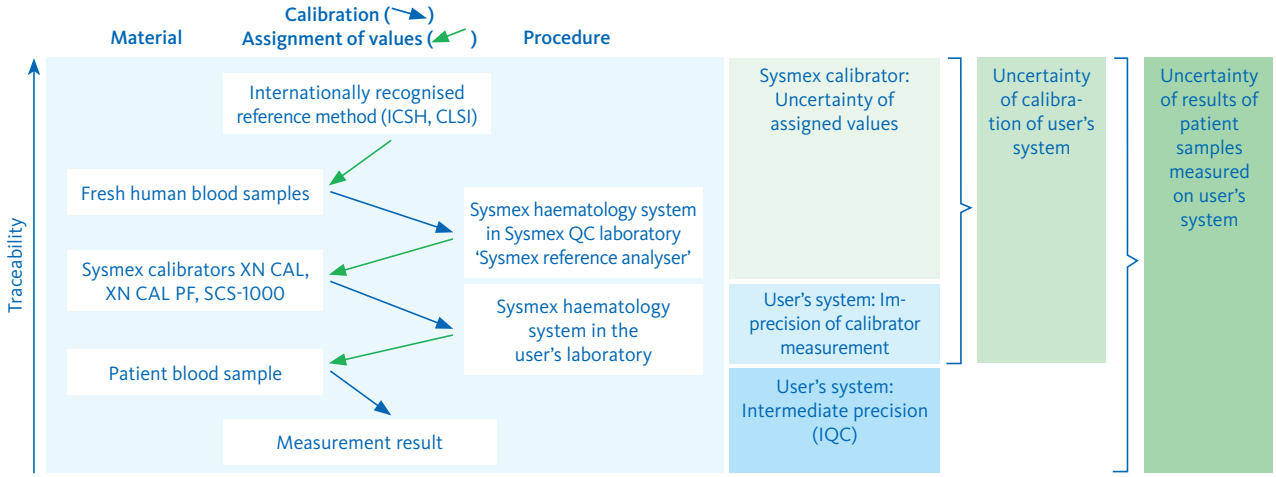

Fig. 3 *Uncertainty components along the traceability chain and combined uncertainty of results of patient samples*

Accreditation

International Standard ISO 15189 [4], which is based on International Standards ISO 9001 and ISO/IEC 17025, is the general basis for the accreditation of medical laboratories. This standard requires medical laboratories to document the metrological traceability of equipment calibration and to determine the uncertainty of their results. Your accreditation agency will be able to advise you in detail of the requirements pertaining to your accreditation.

The calibrators XN CAL, XN CAL PF and SCS-1000 for your Sysmex haematology system and the data provided with these products support you in satisfying the requirements of ISO 15189 pertaining to metrological traceability and uncertainty: The use of XN CAL, XN CAL PF and SCS-1000 will enable you to ensure the traceability of the results of your haematology system to international reference methods. And the uncertainty of the assigned values of these calibrators is part of the overall uncertainty that you calculate for the results of your patient samples. We will be pleased to send you documents on the traceability and uncertainty data for XN CAL, XN CAL PF and SCS-1000 on request.

References

[1] JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM).

[2] JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM, 3rd edition).

[3] ISO 17511:2003 In vitro diagnostic medical devices – Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials.

[4] ISO 15189:2012 Medical laboratories – Requirements for quality and competence.

Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany · Phone +49 40 52726-0 · Fax +49 40 52726-100 · info@sysmex-europe.com · **www.sysmex-europe.com**
You will find your local Sysmex representative's address under www.sysmex-europe.com/contacts

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