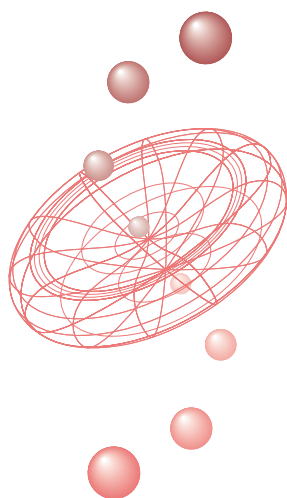


REVIEW ARTICLE



SERIES

16

Metrological Traceability of Values Assigned to Sysmex SCS-1000 Haematology Calibrator

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INTRODUCTION

This article describes the traceability chains of values assigned to the Sysmex SCS-1000 haematology calibrator which are required by ANNEX I A 3 of the *in vitro* diagnostic (IVD) Directive¹ and defined in the subclauses of the new standard prEN ISO 17511 currently in preparation and at the Committee Draft stage².

CALIBRATION HIERARCHY

According to VIM: 1993³) calibration is a “set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards”. Implicit in this definition is the need for a reference measurement procedure to assign a value to a calibrator. Within the definition of reference measurement procedures are different orders. The highest metrological order is the Primary Reference Measurement Procedure (Definitive Method), the next metrological order is the Secondary Reference Measurement Procedure (by metrological institutes) or International Conventional Reference Measurement Procedure (by international scientific

organisations). In haematological circles the latter was previously referred to as the Reference Method. Where neither category exists, for example when a new automated parameter is introduced, the highest metrological order may be the Manufacturer’s Selected Measurement Procedure. Each of these measurement procedures can be used for the assignment of values to a calibrator, the status of these being Primary, Secondary or Manufacturer’s Working Calibrator, respectively.

According to the IVD-Directive¹, “**the traceability of values assigned to calibrators ... must be assured through available reference measurement procedures and/or available reference materials of a higher order**”. There are no primary measurement procedures nor primary reference materials for the components of the blood count. In recent years international conventional reference measurement procedures have been developed for established parameters of the complete blood count. More recently novel parameters have been introduced, e.g. the immature granulocyte count of the XE-2100 where the highest possible metrological order is currently the Manufacturer’s Selected Measurement Procedure. Since the Sysmex SCS-1000 haematology calibrator can be used for WBC, RBC, PLT, HGB and HCT calibration for which there are international conventional reference measurement procedures the traceability chain commences at that level.

NOMENCLATURE AND DEFINITIONS

The following nomenclature and definitions are taken from prEN ISO 17511²⁾ and GUM:1993⁴⁾:

Calibrator

Reference material whose value is used for the independent variable in a calibration function.

Combined standard uncertainty

Standard uncertainty (expressed as a standard deviation) of the result of a measurement when that result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances of these other quantities weighed according to how the measurement result varies with changes in these quantities.

Coverage factor

Numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

Expanded uncertainty

Quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand.

International conventional calibrator

Calibrator material whose value of a quantity is not traceable to the SI but is assigned by international agreement.

International conventional reference measurement procedure

Measurement procedure yielding values that are not traceable to the SI but which by international agreement are used as reference values for a defined quantity.

Manufacturer's selected measurement procedure

A measuring system which is calibrated by one or more primary or secondary calibrators when available.

Manufacturer's standing measurement procedure

A measuring system which is calibrated by one or more of the manufacturer's working calibrators or higher types of calibrator and is validated for analytical specificity.

Manufacturer's working calibrator

Value assigned according to one or more of the manufac-

turer's selected measurement procedures. This calibrator is sometimes called "manufacturer's master calibrator" (or "in-house calibrator"). The calibration material shall have demonstrated commutability as regards the manufacturer's selected measurement procedure and the procedure to be calibrated.

Manufacturer's product calibrator

Value assigned according to the manufacturer's standing measurement procedure and is intended for calibration of the end-user's routine measurement procedure.

Measurement procedure

Set of operations, measuring instrument, reference material or measuring system intended to define, realize, conserve or reproduce a unit or one or more values of a quantity to serve as a reference.

Product calibrator

Calibration material intended for use with the manufacturer's final product.

End-user's routine measurement procedure

A measuring system, often supplied by a manufacturer, and is calibrated by one or more manufacturer's product calibrators.

VALIDATION OF TRACEABLE CALIBRATION

(paragraph 7.4 of prEN ISO 17511²⁾)

Comparison of replicate measurements made by both the reference procedure and the calibrated routine procedure (e.g. for XE-2100 haematology analyser) on a set of actual samples (n>30) typically shows linear regressions as listed in *Table 1*.

UNCERTAINTY OF MEASUREMENT

The uncertainty of measurement is determined and expressed for the assigned target value for each parameter of the Sysmex SCS-1000 haematology calibrator. The principles of calculating the uncertainty are following the "Guide to the expression of uncertainty in measurement" (GUM: 1993)⁴⁾:

Table 1 Validation of traceable calibration

	unit	X mean	Y mean	slope	intercept	multiple R
WBC	×10 ⁹ /L	5.735	5.616	1.0382	-0.3318	0.9994
RBC	×10 ¹² /L	4.967	4.896	1.0240	-0.1901	0.9985
PLT	×10 ⁹ /L	270.9	271.0	1.0040	0.9266	0.9941
HGB	g/dL	11.852	11.773	1.0172	0.2829	0.9989
HCT	%	36.009	35.448	1.0070	0.8122	0.9918

Combined standard uncertainty:

$$U_c(y) = \sqrt{\sigma_1^2 + \sigma_2^2 + \dots + \sigma_n^2}$$

is calculated from the variance of each process step “n” within the traceability chain.

- $U_c(y)$ combined standard uncertainty
- U expanded uncertainty
- σ^2 variance

Expanded uncertainty: $U = U_c(y) \times k$

is calculated with the coverage factor $k=2$, defining approximately the 95% confidence interval about the SCS-1000 calibrator assay value attributed to each quantity.

Typical expanded uncertainties of the Sysmex SCS-1000 haematology calibrator assigned values are (e.g. for XE-2100 haematology analyser):

- WBC = $\pm 0.265 \times 10^9/L$
- RBC = $\pm 0.073 \times 10^{12}/L$
- PLT = $\pm 7.4 \times 10^9/L$
- HGB = $\pm 0.12 \text{ g/dL}$
- HCT = $\pm 0.91 \%$

Note: the numerical value for the expanded uncertainty is the result of an error propagation calculation and characterises the dispersion of the assigned target value taking the total error of the calibration process into account. Therefore the values may differ according to the analyser model used. The valid figures are given for each model on the Sysmex SCS-1000 haematology calibrator assay sheet.

TRACEABILITY CHAINS

The traceability chain for the WBC/RBC count is given in **Fig. 1**, for the PLT count in **Fig. 2**, for the HGB determination in **Fig. 3** and for the HCT determination in **Fig. 4**. The traceability chain is referring to literature and documents which are listed in **Table 2**.

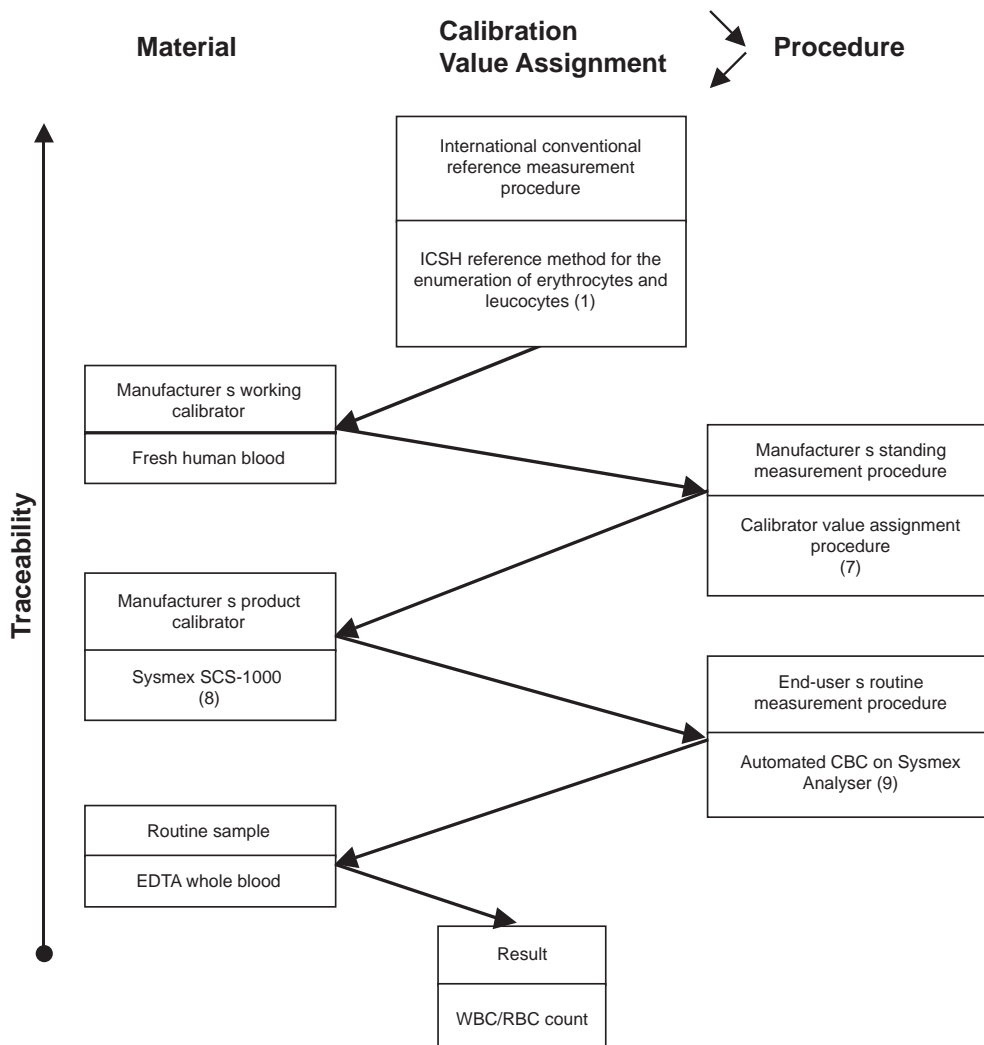


Fig. 1 Traceability chain for WBC/RBC count calibration (subclause 5.4 of prEN ISO 17511²⁾)

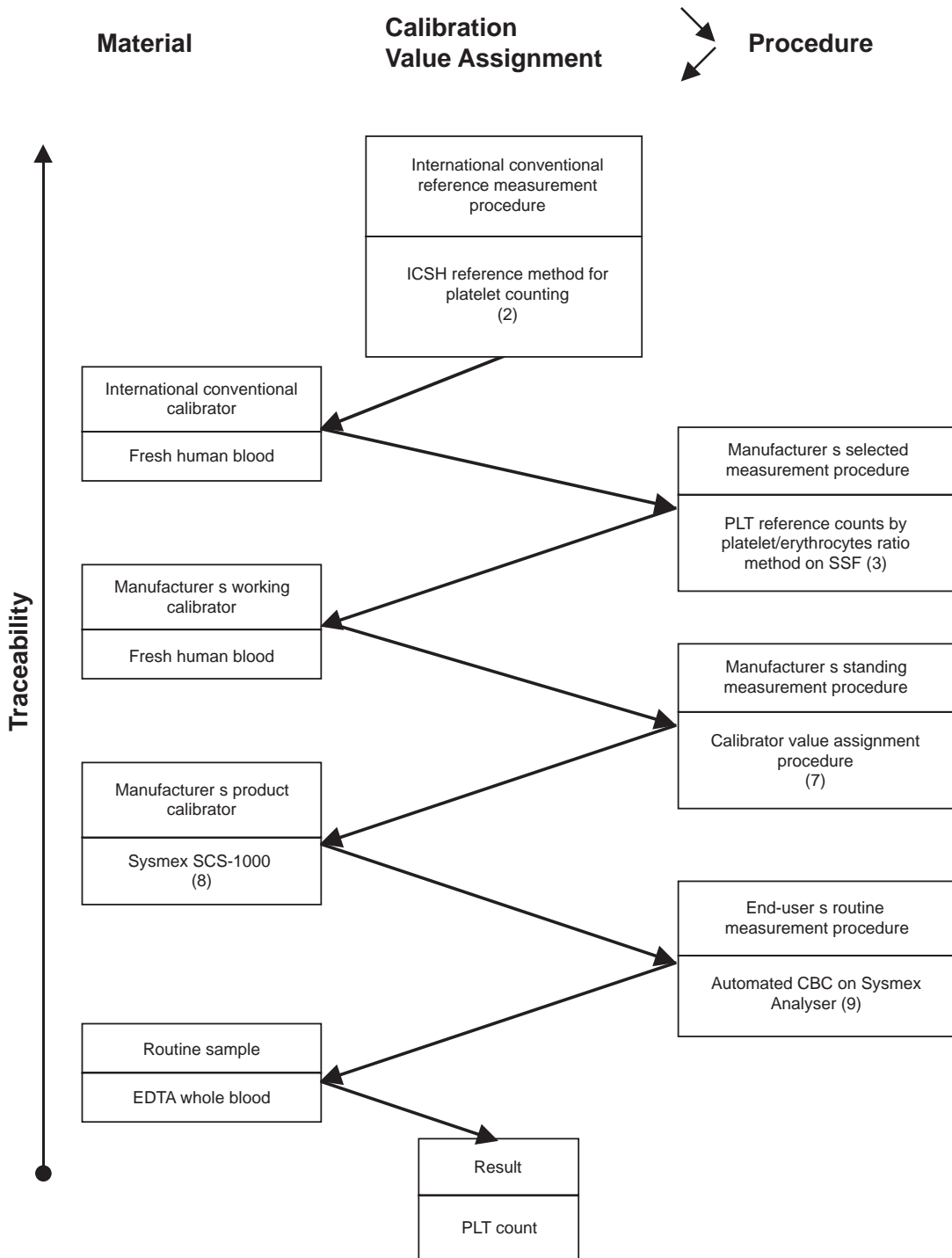


Fig. 2 Traceability chain for PLT count calibration (subclause 5.3 of prEN ISO 17511²⁾)

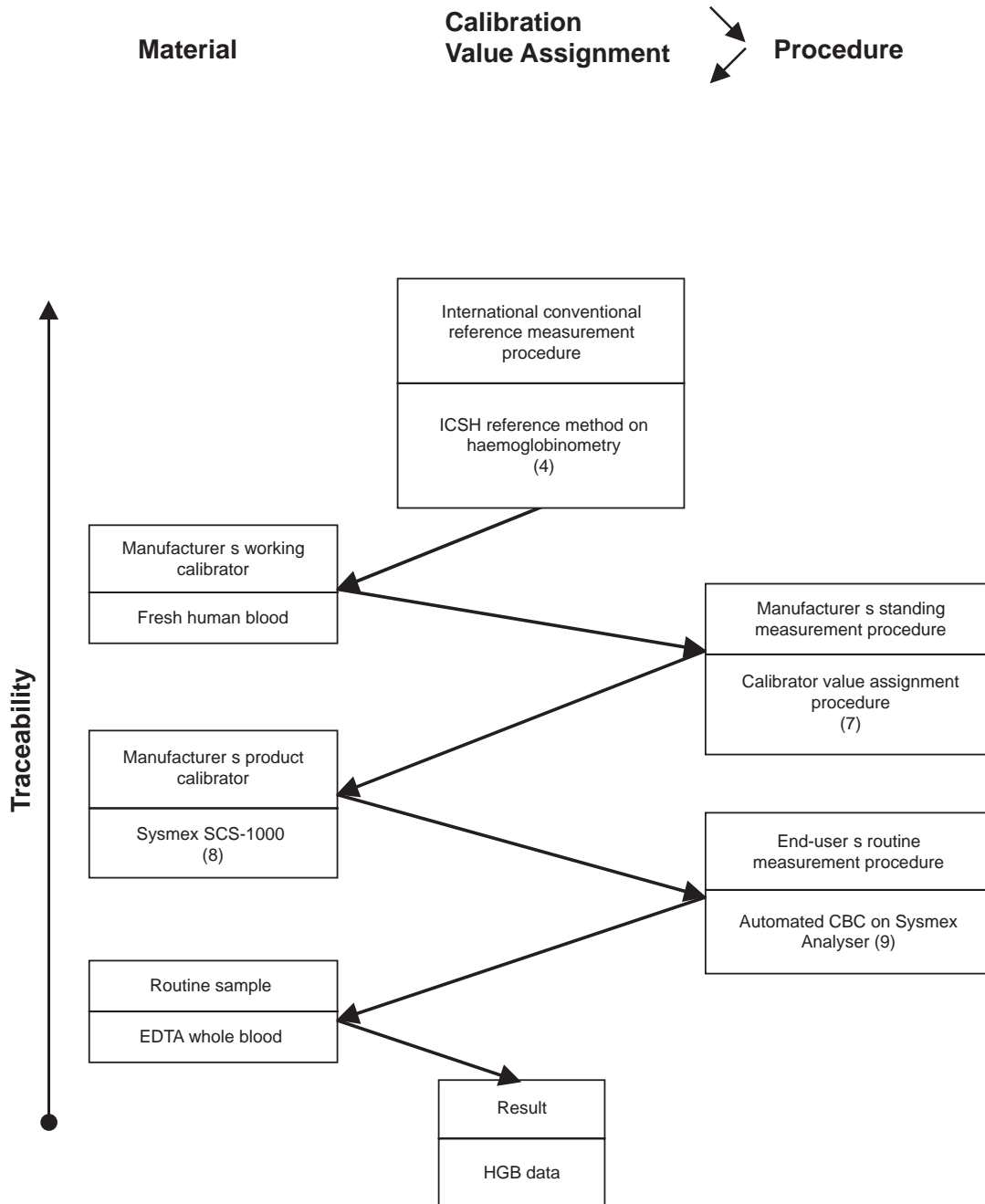


Fig. 3 Traceability chain for HGB calibration (subclause 5.4 of prEN ISO 17511²⁾)

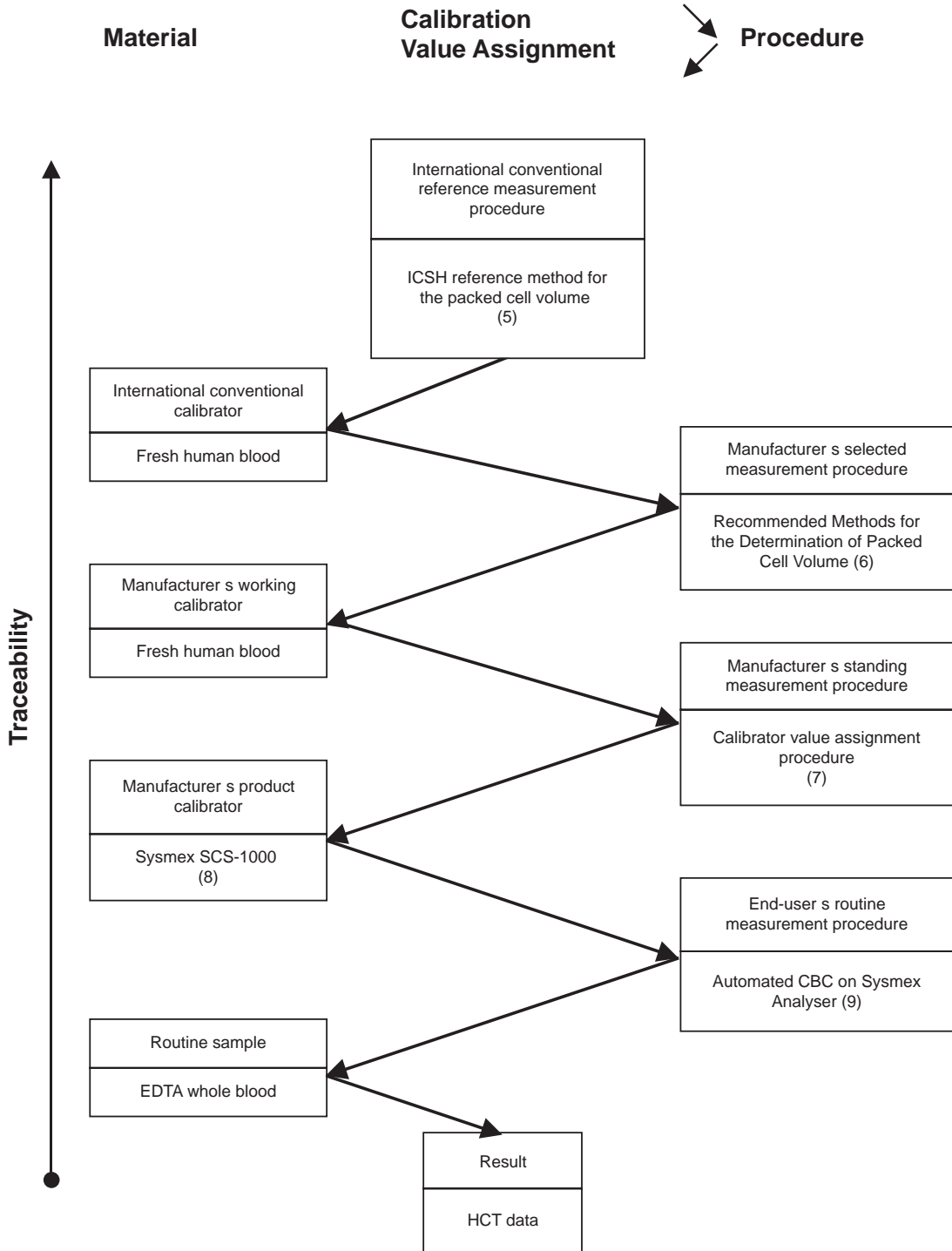


Fig. 4 Traceability chain for HCT calibration (subclause 5.3 of prEN ISO 17511²⁾)

Table 2 Reference literature in the Sysmex SCS-1000 traceability chains

No	Title	Authors	Published in / Version
1	Reference method for the enumeration of erythrocytes and leucocytes	ICSH Expert Panel on Cytometry	Clin Lab Haem, 16: 131-138, 1994.
2	Platelet counting by the RBC/platelet ratio method – A reference method	ICSH Expert Panel on Cytometry ISLH Task Force on Platelet Counting	Am J Clin Pathol, 115: 460-464, 2001.
3	Evaluation of a prototype for reference platelet counter	Lewis S.M., et al.	J Clin Pathol, 43: 932-936, 1990.
4	Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemoglobinocyanide standard (4th edition)	ICSH Expert Panel on Haemoglobinometry	J Clin Pathol, 49: 271-274, 1996.
5	Recommendations for reference method for the packed cell volume (ICSH Standard 2001)	ICSH Expert Panel on Cytometry	Lab Hematol, 7: 148-170, 2001.
6	Recommended methods for the determination of packed cell volume	ICSH Expert Panel on Blood Cell Sizing	WHO LAB/80.4
7	Outline of the value assignment procedure for the SCS-1000 Haematology calibrator	Sysmex Europe GmbH	Norderstedt, Germany, 27-May-2002
8	SCS-1000 Sysmex calibrator system product calibrator for Sysmex haematology analysers	Sysmex Europe GmbH	Norderstedt, Germany, 27-May-2002
9	Instructions for use for Sysmex automated haematology analyser: Chapter 6: sample analysis; analysis mode procedures	Sysmex Corporation	Kobe, Japan release date

References

- 1) Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices (Official Journal of the European Communities L 331/1, 7. 12. 98).
- 2) In Vitro Medical Devices - Measurement of Quantities of Biological Origin - Metrological Traceability of Values Assigned to Calibrators and Control Materials. prEN ISO 17511, Geneva, International Organization for Standardization (ISO), December 2001.
- 3) International Vocabulary of Basic and General Terms in Metrology (VIM), 2nd edition. Geneva, International Organization for Standardization (ISO), 1993.
- 4) Guide to the Expression of Uncertainty in Measurement (GUM), Geneva, International Organization for Standardization (ISO), 1993.