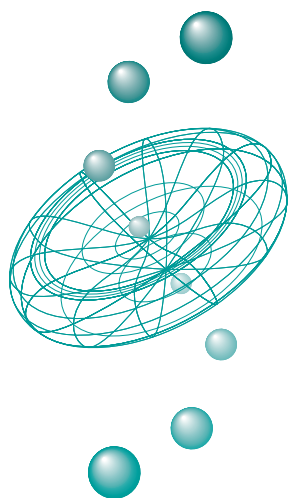


**REVIEW**  
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## Traceability of Haematology Results – An Industry View

**M. KUTZNER and R.M. ROWAN**

Sysmex Europe GmbH, Bornbarch 1, 22848  
Norderstedt, Germany.

### Key Words

*In vitro* Diagnostic (IVD) Medical Device Directive, Metrological Traceability,  
Uncertainty of Measurement, Calibrator, Trueness Control Material

### THE TERMINOLOGICAL DILEMMA

One of the important goals in laboratory medicine is the achievement of comparability of measurable quantities over time and space either by the same measurement procedure or by different procedures measuring the same quantity. Such comparability of data on a global scale would contribute to improvements in health care and in the interpretation of clinical studies undertaken in different locations or times. A way to overcome the current disparities within routine methods is to establish traceability of results for all measurement procedures to reference systems (reference materials and/or reference measurement procedures) of higher metrological order. This currently is not possible for all analytes.

The increasing regulation which has developed over recent years has created, in its wake, a new and important vocabulary using words whose definition has subtly altered from that in common usage and even inventing new words. It is increasingly important that such words are accorded internationally recognised standardised definitions. With regulation comes the force of law. Interpretation must therefore be unequivocal and this can only be achieved following global harmonisation of definitions. It is encouraging that through the Agreement on Technical Co-operation between ISO (International Organization for Standardization) and CEN (European Committee for Standardization)<sup>1)</sup>, the Vienna Agreement, the basis for such harmonisation already exists.

'Traceability' although not a new word is increasingly

used in laboratory practice. Here we are on secure ground since its definition by the ISO in the International Vocabulary of Basic and General terms in Metrology (1993)<sup>2)</sup> is included unchanged in the definitions of the CEN<sup>3)</sup> and the National Committee for Clinical Laboratory Standards (NCCLS)<sup>4)</sup> in the USA. Traceability is thus defined as the 'property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties'. A traceability chain is created. The objective is to imbue the calibrator with the same degree of trueness as the reference measurement procedure or the reference material. The word 'trueness' is defined as 'the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value' (ISO 3534-1-3.12<sup>5)</sup>). Accuracy, on the other hand is defined as the 'closeness of the agreement between the result of a measurement and a true value of the measurand' (VIM93-3.5<sup>2)</sup>). In the developing (ISO/CD 17511<sup>6)</sup>) the concept of 'accuracy of measurement' relates both to trueness of measurement and precision of measurement. The IVD-Directive<sup>7)</sup>, on the other hand, refers to specific analytical performance characteristics and uses the term "accuracy" and "trueness" synonymously. Büttner<sup>8)</sup> (*Fig. 1*) has presented a classification of analytical performance characteristics and techniques for their evaluation. There is an important difference between 'trueness' and 'accuracy'. The definition of 'uncertainty of measurement' from the same source (VIM, 1993<sup>2)</sup>) is also widely defined as a

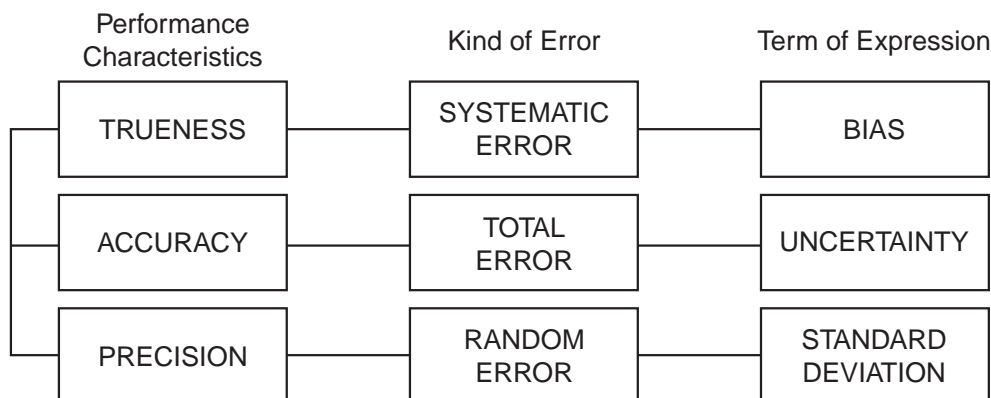


Fig. 1 Expression of analytical specific performance characteristics (from Büttner, 1994<sup>8)</sup>)

‘parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand’. All laboratory tests are subject to uncertainties inherent in the test or errors arising during performance. Although uncertainties arise at three levels, technical (analytical), biological and nosological (Büttner, 1993<sup>9)</sup>), these are additive and give total uncertainty, it is expedient to develop individual models for each. A numerical value on analytical uncertainty can then be determined to support calibrator traceability. When judging the clinical utility of a parameter, however, all three levels must be quantified.

The *In vitro* Diagnostic (IVD) Medical Device Directive<sup>7)</sup> of the EU (Directive 98/79/EC) requires, in Annex I.A.3, that ‘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 higher order’. This means, that a calibrator’s assigned value must be systematically derived from reference materials or methods in an unbroken chain of comparisons. This concept raises another term requiring definition, i.e., commutability. This is defined as ‘the degree to which a material yields the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material’ (CEN EN 12287: 1999, 3.5<sup>10)</sup>). The NCCLS description (NCCLS C37-A<sup>11)</sup>) perhaps clarifies the process by stating that ‘Validation of commutability is therefore demonstrated when the reference material yields a consistent numerical or ratiometric relationship for the among-methods results, when compared to the among-methods numerical relationship (for the same measurement methods) established with the relevant routine test samples’.

It has long been recognised by manufacturers, laboratory professionals and quality assessment organisations that significant methodological / instrumental test result differences occur. In the laboratory this has necessitated the

use of method/ instrument specific reference ranges while the problem has been addressed in external quality assessment organisations by creating peer-group grading for such different laboratory methods. These strategies have worked satisfactorily in the past and still do. Whether or not this continues to be permissible under the IVD Directive<sup>7)</sup> remains to be tested. Traceability and commutability will increase in importance in the coming years.

Analysis of the IVD Directive reveals aspects arising from definitions and from regulations which will necessitate decisions by manufacturers which are urgent and costly.

## STANDARDS DEVELOPMENT IN SUPPORT OF THE IVD DIRECTIVE

\* Acronyms attached to the ISO numerical system: CD equals Committee Draft; DIS equals Draft International Standard and FDIS equals Final Draft International Standard. These relate to stages in the preparation of a standard and where present indicate that full ISO standard status does not exist.

A new standard for the traceability of calibrators for *in vitro* diagnostic medical devices (ISO/CD 17511<sup>6)</sup>) is currently in preparation and is at the Committee Draft stage\*. This is being prepared under a mandate initially given to CEN/CENELEC by the European Commission and the European Free Trade Association and supports an Essential Requirement of the EU IVD Directive<sup>7)</sup>. This document explicitly refers to ‘metrological traceability’ and to ‘calibrators’ and ‘control materials’. Metrology is defined as the science of measurement. The document is being processed in accordance with the Vienna Agreement between ISO (ISO/TC 212, Working Group 2) and CEN (CEN/TC 140) in parallel under CEN lead and is currently submitted to Parallel Enquiry. Although the Committee Draft appears to have been widely circulated, it must be emphasised that it must not be considered to be a Standard at this stage. It remains subject to

change without notice.

The principles embodied in the document, however, must be carefully studied since they may have far-reaching implications for the instrument and kit manufacturer.

## METROLOGICAL TRACEABILITY

The concept of metrological traceability is firmly embedded in the IVD Directive<sup>7)</sup> and ensuring traceability is an Essential Requirement. Traceability is not simply a synonym for accuracy. From an industry stand-point there are important implications for the assignment of values to calibrators and control materials. Traceability of the assigned value of a calibrator is systematically derived from higher order metrology through an unbroken chain of reference measurement procedures and reference materials each step in the chain having stated uncertainties. Suitable 'transfer protocols' must be in place to accomplish this. A 'transfer protocol' is defined as a 'detailed description for assigning a value of a quantity to a reference material using a specified sequence of measurement procedures calibrated by higher-order reference materials for the same type of quantity' (Definitions Used in ISO/TC 212 and CEN/TC 140 Documents, 1999<sup>3)</sup>). The Directive refers to 'available' reference measurement procedures and 'available' reference materials when dealing with assignment of values to calibrators and control materials. This is useful laxity in the Directive but again definitions must be consulted. A reference measurement procedure is defined in EN 12286 (1998)<sup>12)</sup> as a 'thoroughly investigated measurement procedure shown to yield values having an uncertainty of measurement commensurate with its intended use, especially in assessing the trueness of other measurement procedures for the same quantity and in characterising reference materials'. Use of the word 'trueness' in the definition should be noted. Definitions from other organisations use the word 'accuracy' (e.g., ICSH<sup>13)</sup>, NCCLS<sup>4)</sup>, IVD Directive<sup>7)</sup>). If one compares the definitions of the two terms (see Introduction) significant differences become apparent. An hierarchy of reference measurement procedures exists which in the manufacturer's context includes (1) primary (usually international), (2) secondary (usually national), (3) local (manufacturer's selected measurement procedure) and (4) working (manufacturer's routine measurement procedure). A parallel hierarchy exists for calibrators and control materials. Manufacturers have traditionally followed such practice. The new concept is quantitation of the uncertainty at each level in the traceability chain.

Traceability of a value assigned to a calibrator or a control material is therefore established by a series of comparative measurements using reference measurement procedures and / or reference materials, as before, in a route of decreasing hierarchical order (*Fig. 2*). When primary or secondary calibrators are not available, the traceability route begins at a lower level, e.g. at the manufacturer's selected measurement procedure. In the case where a manufacturer develops a new laboratory test and defines the measured quantity by an in-house procedure, this procedure will form the top of the traceability route. Traceability routes, therefore, may, be of different

lengths. Since each link in the route contributes to the uncertainty of the result, as many steps as possible should be avoided. It would be ideal to measure routine samples directly by use of a primary reference procedure, omitting all in-between steps of the traceability route. This is, of course, not a practical possibility.

Although the concept of the traceability chain is quite clear, a number of challenges face calibrator traceability. In this respect the calibrator material, as for all measured quantities, must be clearly defined and include the intended use in medical decision making. ISO/CD 17511<sup>6)</sup> suggests the following details: (1) intended use regarding a medical decision, (2) biological system and component to be characterised, (3) kind of quantity, and (4) unit of measurement. A major difficulty relates to the inhomogeneity of analytes. Few analytes consist of a single well-defined chemical substance. More often the target analyte consists of a group of substances each component of which will convey different pathophysiological connotations and which will behave differently in different measuring systems. Again ISO/CD 17511<sup>6)</sup> proposes a working classification to deal with this. This is a much greater problem in clinical chemistry than in haematology.

## UNCERTAINTY OF MEASUREMENT

The ISO definition of uncertainty of measurement has already been quoted (*vide supra*). According to classical metrological thinking, a measurement result is only complete when accompanied by a quantitative statement of its uncertainty. Uncertainty is required to decide if a result is adequate for its intended purpose and to establish its consistency with other similar results. The primary source of information on uncertainty is to be found in ISO Guide to the Expression of Uncertainty in Measurement (1993)<sup>14)</sup> but is highly statistical in its presentation. ISO/CD 17511<sup>6)</sup> recommends that the principles given in the Guide should be followed. These recommendations include three further complex terms which require definition. The first is 'expanded uncertainty' which is the 'quantity defining an interval about the result of an measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand'. The second is 'coverage factor' which is defined as the 'numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty'. Finally 'combined standard uncertainty' is the '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'. Uncertainties are classified according to the method used to estimate their numerical value; Type A which are evaluated by statistical methods and Type B which are evaluated by probability distributions or other means. This model of uncertainty of measurement while an internationally recog-

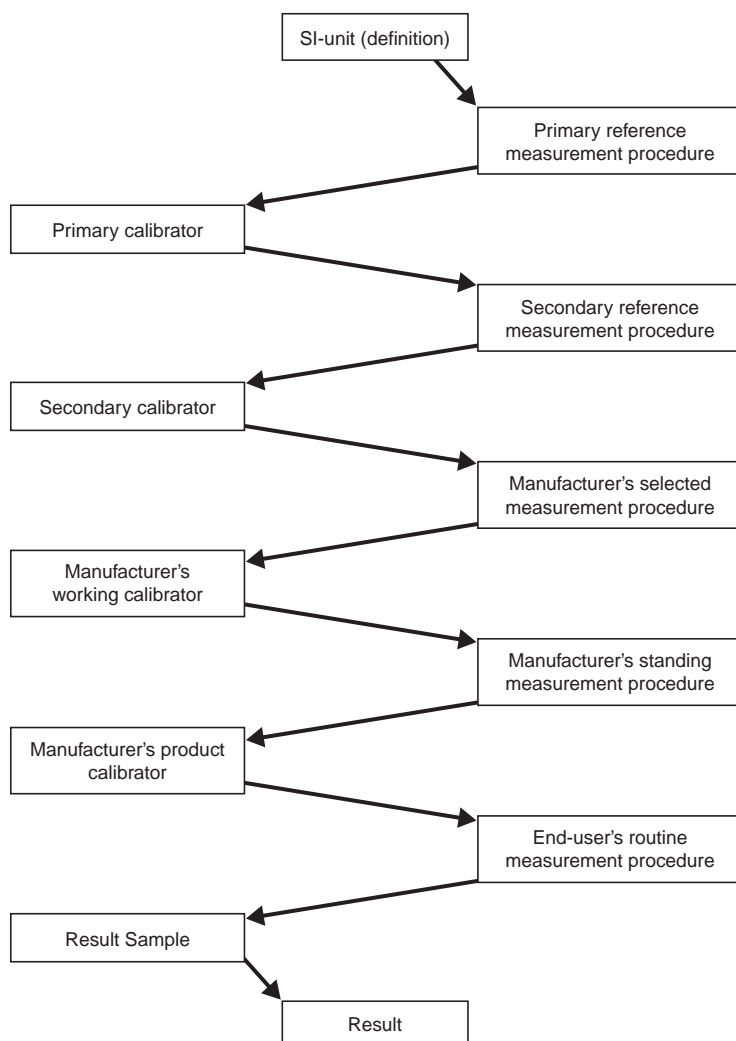


Fig. 2 Calibration hierarchy and metrological traceability chain (adapted from <sup>6)</sup>)

nised metrological standard is not currently used by laboratory professionals nor the IVD industry.

In another developing document, ISO/FDIS 15189 (Quality Management in the Medical Laboratory)<sup>15)</sup> there can be found the statement (paragraph 5.6.2): 'The laboratory shall determine the uncertainty of its measurements, where relevant and possible. Uncertainty components which are of importance shall be taken into account'. This implies, first, that the determination of the uncertainty of measurement is a laboratory responsibility and, secondly, that its quantitation may be impossible or of no relevance. Although these documents are intended for different constituencies within laboratory medicine, the concepts embodied are at variance one from the other. There is no reference to uncertainty of measurement in EN 45001 (General criteria for the operation of testing laboratories<sup>16)</sup>) however, the subject is referred to in ISO/IEC 17025, 1999 Para. 5.4.6<sup>17)</sup>.

While standard deviations usually can be easily determined (series of measurements to detect random error), bias (to detect systematic error) can be only determined if

a true value exists. For many haematology parameters, however, estimation of a true value is not yet possible, e.g., for the components of the differential leukocyte count. For concentration measurements of simple molecules in serum this model of uncertainty is probably applicable and makes sense. The classical metrologist, however, recommends the model for all laboratory testing ignoring the fundamental differences of measurement applied in haematology, coagulation, urinalysis and in the detection of infectious diseases (yes or no decisions). It is, however reassuring to note that exclusions exist in ISO/CD 17511<sup>6)</sup>.

In the context of discussing metrological uncertainty of measurement of tests, the question arises on the impact on medical decision making when reporting those. The values for metrological uncertainty often become trivial, when considered in the contexts of biological variability and nosological influences that require to be taken into account by clinicians when interpreting patient test results.

## IMPACT ON INDUSTRY

The implications of the metrological traceability requirement contained in the IVD Directive are far-reaching for global industry. Some of the requirements are still uncertain and in the face of international politics, and the competing priorities of an industry under heavy cost constraints additional effort will require clear and cogent justification, clinical as well as analytical.

The ISO model of uncertainty<sup>14)</sup> has not been used to characterise the total error in IVD calibration. At Sysmex, currently, for specifying random error, precision data are determined (usually repeatability of a normal sample). Accuracy is determined as the bias to a selected in-house reference method. With our unique expertise and our appreciation of the importance of these in-house measurements, we take extra care to assure that interference and environmental factors are minimised. This translates to assay procedures which show generally lower imprecision than end-users can achieve in routine practice. As such, our in-house measurement procedures, using the identical methodological principles as in routine medical laboratory practise, are actually higher order procedures. So, even if there was no additional calibration above the in-house procedure, the values assigned to calibrators and control material would still have traceability. This approach is not excluded by ISO/CD 17511<sup>6)</sup>.

It makes sense to implement metrological traceability for those parameters that have reference materials and reference methods, provided use of the parameters is clinically justifiable. Then, once a workable infrastructure is in place, the system can be expanded to cover other parameters.

As an example for calibrator traceability in Sysmex, the traceability route of the Sysmex calibrator SCS-1000 for red cell counting is shown (Fig. 3). In the absence of a primary or secondary calibrator, the highest order reference is the ICSH Reference method for the enumeration of erythrocytes and leucocytes (1994)<sup>18)</sup>. This method was applied by Sysmex on the Reference Instrument for RBC counting. The Reference Instrument is used to calibrate the Standard Instrument by using fresh human blood (Manufacturer's working calibrator). The Standard Instrument is a routine haematology analyser which is used to determine the specific RBC calibration assay (Manufacturer's standing measurement procedure) for the SCS-1000 calibrator (Manufacturer's product calibrator).

Industry has to realise that the word 'traceability' is not merely a synonym for accuracy. Models currently used to determine the total error in IVD calibration do not follow the ISO model of uncertainty of measurement. Therefore, for industry, additional effort will be required to determine data on uncertainty of calibration proce-

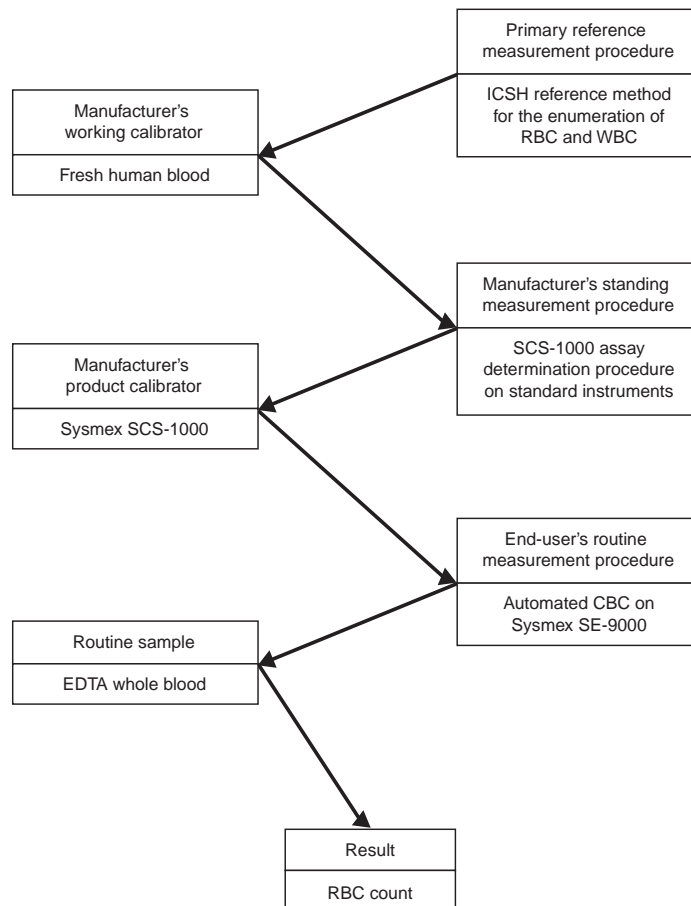


Fig. 3 Traceability chain for RBC count calibration

dures. Is the current documentation sufficient? Do protocols and procedures for assay value determination require to be altered? Is further investment in the development of additional reference materials required? Will it become necessary for the costly outsourcing of assay value determination to independent, accredited reference laboratories?

Traceability is not only required for calibrators but also to trueness control materials such as control blood used in laboratory haematology. Precision control materials are excluded from the requirement. It is the intention of the manufacturer to provide customers with a stable, convenient and low-cost tool to verify the performance of the analyser. To address this fully, additional analytical features may become necessary in the design of control material manufacture which will result in system specific values. Measuring such a product by reference methods will be nothing more than an expensive analytical exercise. Any relationship between reference values and system-specific assigned values will be purely coincidental. All laboratories will get added cost for values which are of no practical use.

The transition period of the European Union's IVD Directive<sup>7)</sup> is officially under way, and CE marking of products becomes mandatory in beginning of December 2003. Industry must therefore make haste to develop strategies for achieving compliance.

## CLINICAL IMPACT

It is the support of medical decision making and not meeting metrological requirements that is the *raison d'être* of laboratory medicine. The IVD Directive<sup>7)</sup> acknowledges this in Essential Requirements (A. General Requirements: Para 1) which states 'when used under the conditions and for the purposes intended, they will not

compromise, directly or indirectly, the clinical condition or the safety of the patients ....'. Consideration of the utility of a laboratory test therefore must include analytical, biological and nosological uncertainties and their effect on calibrators and control materials. The impact of the Directive may be to generate intense activity in the field of reference method procedures and reference materials, both revision of the existing and creation of the new in an attempt to achieve compliance. The end result would be change in calibration processes with an inevitable change in reference ranges and medical decision limits. Unless well publicised, this could lead to serious medical error. In a sense, it is comforting that the Directive states only that 'routine methods need to be traceable to "available reference measurement procedures and/or available reference materials of a higher metrological order" '. In the same way ISO/CD 17511<sup>6)</sup> acknowledges situations where no higher-order methods or materials exist.

The ISO/CD 17511<sup>6)</sup> document excludes discussion of all items that are not strictly 'metrological'. Thus, a prerequisite for making correct medical decisions is that the actual pre-analytical, analytical and post-analytical conditions are considered and the errors in these procedures are known. Many factors are involved such as point in disease process, method of collecting and handling patient specimens, analytical procedure and methods of expressing and transmitting results to clinicians.

## LIST OF REFERENCE LITERATURE

Reference literature which can be used to prove traceability of measurement in laboratory haematology is listed in *Table 1*.

*Table 1 Reference literature in laboratory haematology*

Title	Authors	Published in
Reference method for the enumeration of erythrocytes and leucocytes	ICSH Expert Panel on Cytometry	Clin lab Haemat, 16: 131-138, 1994.
Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemiglobincyanide standard (4th edition)	ICSH Expert Panel on Haemoglobinometry	J Clin Pathol, 49: 271-274, 1996.
Lauryl sulphate haemoglobin: a non hazardous substitute for HiCN in haemoglobinometry	Lewis S M, et al.	Clin lab Haemat, 13: 279-290, 1991.
Recommended methods for the determination of packed cell volume	ICSH Expert Panel on Blood Cell Sizing	WHO LAB/80.4
Proposed reference method for reticulocyte counting based on the determination of the reticulocyte to red cell ratio	ICSH Expert Panel on Cytometry	Clin lab Maemat, 20:77-79, 1998.
Coincidence correction in red blood cell counting	Lewis S M, et al.	Phys Med Biol, 34: 1239-1246, 1989.
Standardisation for haemocytometry	Lewis S M, et al.	Laboratory Practice, 38: 68-69.

## CONCLUSIONS

Several messages emerge from careful scrutiny of the IVD Directive<sup>7)</sup> and its associated publications. The first and perhaps the most important is that the Directive does not mandate the development of new reference measurement procedures nor reference materials. Any decision to develop these must be dictated by the requirements of the medical community and by practicalities. The benefits of applying traceable reference materials must be evaluated in relation to medical outcomes. Cost-effectiveness is an important consideration and must be demonstrable. Currently there are many standardising bodies with resulting duplication of effort. A global infrastructure requires to be created; not necessarily the creation of a single authority but certainly a global organisation aware of what is going on world-wide. Existing organisations might then enter into mutual recognition schemes provided guidance was available on the interpretation of relevant regulations and standards. There is urgent need to start planning such solutions. Precipitate uncoordinated action must be avoided, however, since escalation of medical error would result. To achieve this, close co-operation between laboratory professionals, public health authorities and the IVD industry must exist. International politics have no place in the process.

### References

- 1) *International Organization for Standardization (ISO): European Committee for Standardization (CEN). Guidelines for TC/SC Chairmen and Secretariats for implementation of the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). Revision, 1995.*
- 2) *International Vocabulary of Basic and General Terms in Metrology (VIM), 2nd edition. Geneva: International Organization for Standardization (ISO), 1993.*
- 3) *Compendium of nomenclature and definitions used in ISO/TC 212 and CEN/TC 140, ISO/TC 212. NCCLS (National Committee for Clinical Laboratory Standards), 940 West Valley Road, Suite 1400 Wayne, PA, 19087-1898 USA, 1999.*
- 4) *National Committee for Clinical Laboratory Standards (NCCLS). Terminology and Definitions for Use in NCCLS Documents; Approved Standard. NCCLS Publication NRSC8-A [ISBN 1-56238-359-0]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087-1898, USA, 1998.*
- 5) *Statistics-Vocabulary and symbols-Part 1: Probability and general statistical terms. ISO 3534-1, Geneva: International Organization for Standardization (ISO), 1993.*
- 6) *In Vitro Medical Devices-Measurement of Quantities of Biological Origin-Metrological Traceability of Values Assigned to calibrators and Control Materials, ISO/CD 17511, Geneva: International Organization for Standardization (ISO), 2000.*
- 7) *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, 7.12.98).*
- 8) *Büttner J: Reference Materials and Reference Methods in Laboratory Medicine: A Challenge to International Cooperation, Eur J Clin Chem Clin Biochem, 32,8: 571-577, 1994.*
- 9) *Büttner J: Evaluation of Diagnostic and Prognostic Measures, in Evaluation Methods in Laboratory Medicine, ed. R Haeckel, Weinheim, VCH, 1993.*
- 10) *In Vitro Diagnostic Medical Devices-Measurement of Quantities in Samples of Biological Origin-Description of Reference Materials. ISO/DIS 15194 (formerly EN 12287: 1999) Geneva: International Organization for Standardization, 1999.*
- 11) *National Committee for Clinical Laboratory Standards (NCCLS). Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures: Approved Guideline, NCCLS document C37-A [ISBN 1-56238-392-2]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1999.*
- 12) *In Vitro Diagnostic Systems-Measurement of Quantities in Samples of Biological Origin-Preparation of Reference Measurement Procedures, ISO/DIS 15193 (formerly EN 12286: 1998), Geneva: International Organization for Standardization (ISO), 1999.*
- 13) *International Council for Standardization in Haematology (ICSH), Rules and Operating Procedures, ICSH Secretariat, Leuven, Belgium, 1991.*
- 14) *Guide to the Expression of Uncertainty in Measurement (GUM), Geneva, International Organization for Standardization (ISO), 1993.*
- 15) *Quality management in the medical laboratory, ISO/FDIS 15189, Geneva, International Organization for Standardization (ISO), 2000.*
- 16) *General criteria for the operation of testing laboratories, EN 45001, Brussels, CEN/CENELEC, 1989.*
- 17) *General requirements for the competence of testing and calibration laboratories, ISO/IEC 17025, Geneva, International Organization for Standardization (ISO), 1999.*
- 18) *Reference method for the enumeration of erythrocytes and leucocytes (ICSH): Clin Lab Haemat, 16: 131-138, 1994.*