

## Actual State of International Standardization and External Quality Assurance for Blood Cell Counts



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In hematology testing using hematology analyzer, there are no internationally certified reference materials. Therefore, an internationally recognized reference method that enables clarification of traceability of measurements is necessary because fresh blood must be used for the assurance of accuracy in hematology testing. Guidelines for the reference method are being formulated in an international framework. External quality assurance provides objective information for evaluation of the reliability of a measurement system, and standardization of the implementation of external quality assurance is critical.

### Introduction

Hematology testing plays an important role in routine medical care, and in particular a Complete Blood Count (CBC) and differential white blood count are essential to almost all types of clinical diagnosis. For this reason, a high level of accuracy is demanded. To ensure that high-accuracy, high-quality medical service is provided, an increasing number of clinical laboratories have been obtaining certification such as ISO 15189 (Medical laboratories--Requirements for quality and competence). This indicates that there is a demand for laboratory management that conforms to international standardization. This paper gives an outline of traceability in blood count testing, trends in international standardization, international conventional reference measurement procedures, and external quality assurance. It provides an overview of the current state of quality assurance and international standardization of blood count testing that uses a fresh blood sample as a real sample reference material, and touches on some problems in this area.

### Traceability of Blood Count Parameters

The European directive on in vitro diagnostic medical devices (Directive 98/79/EC on in vitro diagnostic medical devices) enacted in 2004 requires that manufacturers of clinical test instruments and reagents ensure that analysis result traceability is maintained by means of suitable standard materials.<sup>[1]</sup> To verify the validity of analysis results, the measurement system is required to be traceable in SI units, and by clarifying traceability, the range of reliability (uncertainty) of

analysis values can be indicated.

In the field of clinical testing, there is often no traceability for SI units because there are no international standards for physical quantities such as mass and distance. In clinical chemical testing, traceability can be made clear by using an internationally Certified Reference Material (CRM). However, in the field of hematology testing, blood cells are the target of analysis, and as there are no stable reference materials that can be used in common for hematology analyzers that are based on a variety of measurement principles and reagent systems, it is necessary to use fresh blood to ensure accuracy. This type of example corresponds to “When there is an international conventional reference measurement procedure (not primary), but no metrological traceability to SI units, and there is no international conventional calibration material” (ISO 17511:2003), and the reference analysis method that assigns values to the real sample reference material is thus critical.<sup>[2]</sup>

An example of a system diagram for complete blood count traceability is shown in Figure 1. The reference analysis methods of the International Council for Standardization in Hematology (ICSH), World Health Organization (WHO), and Clinical and Laboratory Standards Institute (CLSI) are recommended as international conventional reference measurement procedures. As reference measurement procedures for CBC measurement using a hematology analyzer, HORIBA, Ltd. uses the ICSH reference method (single-channel electrical resistance method) for red blood count and white blood count, the CLSI reference method (cyanmethemoglobin

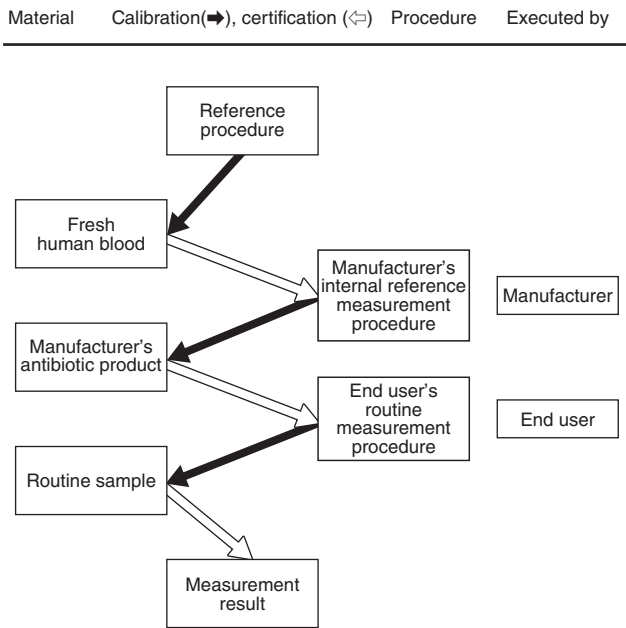


Figure 1 Example of complete blood cell count traceability diagram

method) for hemoglobin concentration, the CLSI reference method (micro-hematocrit method) for hematocrit value, and the WHO method (Brecher-Cronkite method) for platelet count.

### Trends in Blood Cell Count Standardization

Efforts to internationally standardize blood cell count parameters have centered on the ICSH and the CLSI. The ICSH has worked in collaboration with WHO. The ICSH is currently positioned as a standardization working group of the International Society for Laboratory Hematology (ISLH), and collaborates with the CLSI to advance international standardization of reference analysis methods for blood cell count parameters.<sup>[3]</sup> Upon completion of a standardization project, the ICSH normally publishes the results as an academic paper, and posts the content on the ICSH website. The content can be downloaded freely. In Japan, the Japanese Society for Laboratory Hematology (JSLH) is acting in concert with the ISLH and ICSH to address issues in the standardization of hematology test methods. The action policy of the JSLH states “As international conventional reference measurement procedures and reference measurement procedures, the JSLH recommends the reference methods described in documents published by the ICSH, Clinical and the CLSI, and WHO, which are organizations that are working to advance international standardization”. This policy is posted on the JSLH website.

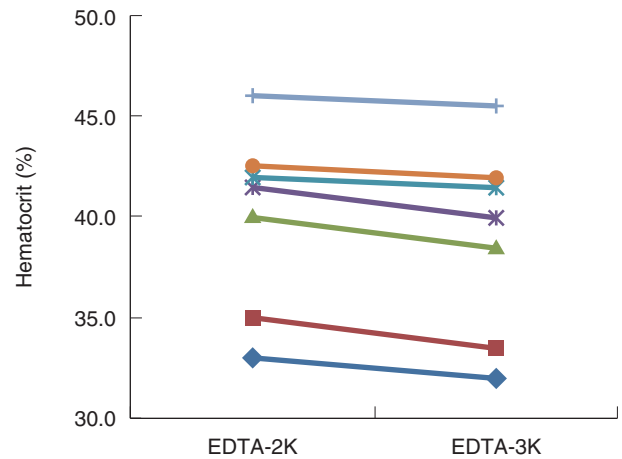


Figure 2 Effects of EDTA salt types (hematocrit values) (Changed from citation<sup>[7]</sup>)

## Overview of International Conventional Reference Measurement Procedures

### Sample collection and anticoagulant

A fresh blood sample collected from a healthy individual is used as a sample. EDTA-2K (1.5 to 2.2 mg/dL blood) or EDTA-3K (1.5 to 2.2 mg/dL blood) is used as the anticoagulant.<sup>[4, 5]</sup> EDTA-2K is widely used in Europe and Japan. EDTA-3K is widely used in North America. It is known that EDTA-3K contracts blood cells more than EDTA-2K and thus a low hematocrit value is indicated. This effect increases with time, hence blood with EDTA-3K is analyzed within 3 hours of collection.<sup>[6]</sup> The ICSH recommends EDTA-2K as an anticoagulant for CBC because it has the least effect. After anticoagulant is added, the sample is stored at 20 ± 2°C and is analyzed within 4 hours. In the author’s own experience as well, when we added EDTA-2K and EDTA-3K to venous blood samples so as to attain a final concentration of 1.5 mg/mL, the sample in which EDTA-3K was used indicated a hematocrit value that was 1.40% (N = 7, p < 0.01) lower than the sample with EDTA-2K. (Figure 2)<sup>[7]</sup> When we varied the concentration of added EDTA, the hematocrit value showed a decline as the concentration increased, and this decline was more prominent in the EDTA-3K samples than in the EDTA-2K samples.

### Hemoglobin concentration

The reference analysis method is the cyanmethemoglobin method based on colorimetric analysis at a measurement wavelength of 540 nm, and the ISCH guidelines specify measurement procedures, standard solution preparation procedures, measurement reagent preparation procedures, and evaluation procedures for manufactured reagents.<sup>[8]</sup> For the standard solution used in colorimetric analysis, the cyanmethemoglobin standard solution (ICSH HiCN Standard) supplied by Eurotrol B.V. is used. This standard

solution cannot be directly used for analysis by a hematology analyzer. It is used as the standard solution for the reference analysis method, and is used to assign values to fresh blood for calibration of manufacturers' internal reference analysis methods.

### Hematocrit value

The hematocrit value is also referred to as PCV (Packed Cell Volume). The reference analysis method is the micro-hematocrit method without consideration given to trapped plasma.<sup>[9]</sup> Trapped plasma is plasma that is trapped between blood cells in the blood cell layer following centrifugation. By contrast, a hematology analyzer multiplies the volume of each red blood cell by the number of blood cell pulses to obtain the hematocrit value. This eliminates the effect of trapped plasma, and the measured value is different from that of the reference analysis method due to the difference of measurement principle.

### Red blood cell and white blood cell counts

The reference analysis method uses a single-channel, electrical resistance type blood cell counter.<sup>[10]</sup> "The assignment of values to fresh blood used for calibrating automated blood cell counters" in the ICSH guidelines specifies methods for assigning values to calibration blood and control blood, methods for determining control limits, and instrument specifications and test methods.<sup>[11]</sup>

### Platelet count

Reference analysis methods include an indirect method (FCM method) and a direct method. The indirect method calculates the platelet count from the proportion of the platelet count to the red blood cell count obtained by immunological Flow Cytometry (FCM) using CD41 and CD61 antibodies marked by fluorescent dye, and from the absolute count of red blood cells obtained using a hematology analyzer. In the direct method, the blood sample is diluted with a 1% solution of ammonium oxalate, and the platelet count is calculated using a calculating board and a phase-contrast microscope.<sup>[12]</sup>

### Reticulocyte count

Reference analysis methods include a visual calculation method using a mirror disk and microscope, and the FCM method. The sample is dyed using new methylene blue. The most recent guidelines describe an FCM method that uses thiazole orange fluorescent pigment for the nucleic acid stain, and there is also text regarding the Immature Reticulocyte Fraction (IRF).<sup>[13]</sup> Although the guidelines cite literature related to the FCM method, there are no detailed descriptions of measurement procedures, scattergram analysis methods, or other techniques.

### Differential white blood count

The reference analysis methods include a visual differential method that uses Romanowski stain on a thin-layer blood smear, and an FCM method that uses monoclonal antibodies and flow cytometry. The most recent guidelines indicate disadvantages of the current visual differentiation method such as poor reproducibility for low proportion cells due to dependence on the subjective judgment of the technician, and the effects of the cell distribution state on the effects of the distribution of the cells in the smear. By contrast, the guidelines state that the FCM method has a much higher number of analysis cells than the visual method and enables precise white blood cell differentiation, and thus is suitable as a reference method for hematology analyzers.<sup>[14]</sup> Although the guidelines cite related literature, there are no detailed descriptions of procedures. The ICSH is currently evaluating an FCM method that uses monoclonal antibodies.

## Blood Cell Count Parameter External Quality Assurance (EQA)

A summary of the External Quality Assurance program for blood cell count parameters that is being implemented in Japan on a nationwide basis is given in Table 1.<sup>[15]</sup> There are additional EQA programs that are implemented on a local basis under the joint or independent sponsorship of medical associations and clinical technologist

Table 1 Nationwide EQA for automated blood cell count parameters

Organization	Sample	Times per year	Number of participating labs*	Survey parameters					Other parameters
				RBC	Hb	Ht	WBC	Plt	
Japanese Association of Medical Technologists	Processed blood	1	3404	○	○		○	○	MCV
Japan Medical Association	Processed blood	1	3140	○	○	○	○	○	
Japan Society of Health Evaluation and Promotion	Processed blood	2	390	○	○	○	○	○	MCV, MCH, MCHC
National Federation of Industrial Health Organization	Processed blood, fresh blood**	1	360	○	○	○	○	○	MCV
Japan Registered Clinical Laboratories Association	Fresh blood***	1	260	○	○	○	○	○	Neut, Lympho (other differential white blood count)

\* Approx. numbers for 2012-2013, \*\* EDTA-2K added after blood collected in transfusion bag containing CPDA, \*\*\* Blood with EDTA-2K added

associations, and most of these use fresh blood. There are also EQA programs in related hospitals and programs implemented by hematology analyzer manufacturers that target users. A related society, the Japanese Society of Laboratory Hematology (JSLH), has been implementing the JSLH 6-company EQA program once every 2 to 4 years since 2001, with the cooperation of six companies.<sup>[16]</sup>

Processed blood and fresh blood are used as test samples in these EQA programs. Chemicals are added to fix and stabilize processed blood in the same way as the control blood used in internal quality control, so storage stability is excellent and it is easy to prepare a large number of samples. Although matrix effects that accompany the fixing process and added chemicals sometimes cause deviations between instruments, using fresh blood similar to routine samples eliminates this concern.

For example, when fresh blood with EDTA added is measured using a precisely calibrated electrical resistance type hematology analyzer and light scattering type hematology analyzer, no differences are observed in the measured values of the two instruments. However, when processed blood is measured, matrix effects cause values that are lower on the light scattering type than on the electrical resistance type.<sup>[3]</sup> Optical principles require that red blood cells be expanded in order to measure red blood cell volume from the cross-sectional area of the forward scattered light, and because fresh blood behaves differently from fixed processed blood when diluent is added, deviations occur due to the measurement principle. Some hematology analyzers expand the blood cells for measurement and some analyzers contract the blood cells for measurement, and the osmotic pressure of the red blood cell diluent varies by analyzer. The osmotic pressure range of red blood cell diluent for hematology analyzers currently on the market is approximately 250 mOsm to 331 mOsm.<sup>[17]</sup>

Regarding samples used for Japanese nationwide EQA, the Japanese Association of Medical Technologists, Japan Medical Association, and Japan Society of Health Evaluation and Promotion use processed blood, the Japan Registered Clinical Laboratories Association uses fresh blood with EDTA-2K added, and the National Federation of Industrial Health Organization uses fresh venous blood collected in a blood transfusion bag containing CPD (citrate phosphate dextrose) with EDTA-2K added after collection. In a survey regarding EQA programs implemented by local medical technologist associations, 44% of the associations use fresh venous blood collected in a blood transfusion bag containing CPD-A with EDTA-

2K added after collection, 30% use fresh blood with EDTA-2K added, 11% use fresh blood with CPD-A added, and 15% use other samples.<sup>[18]</sup>

If the large-scale nationwide EQA program were able to use fresh blood with anticoagulant added, the same as routine samples, matrix effects could be avoided and all models could be evaluated uniformly. However, there is a limit to the amount of blood volunteers can supply, and a large amount of fresh blood material cannot be prepared. Even so, if the reported fresh blood EQA values of each organization were shared and used for evaluation, analysis on a nationwide scale would be possible. The near future will see an increasing need to build a system that standardizes work procedures for sample preparation and other tasks, and allows sharing of data.

## Conclusions

Because there are no internal certified standard materials suitable for blood cell count testing using hematology analyzers, it is necessary to propagate accurate values by using international conventional reference procedures to assign values to fresh blood. The development of reference analysis methods and the drafting of guidelines are being pursued in an international framework, and a sufficient understanding of trends in this area is desirable. Regarding the reference analysis procedures for blood cell count parameters, it is expected that, like platelet count, FCM will become the main method used for white blood cell differentiation and reticulocyte testing due to reasons such as differences in the number of analysis cells, specificity, and objectivity in the visual calculation method and in automated blood cell analysis.

Objective evaluation using EQA has a large role to play in the maintenance and improvement of the reliability of test results, and in appeals made to hematology analyzer manufacturers. To implement EQA in ideal conditions for blood cell test parameters in particular, the standardization of procedures for preparation of distributed samples and analysis methods for reported values must be pursued.

## References

- [1] Nagai Yutaka, Noriyuki Tatsumi, "Differences in the assurance of accuracy between hematology tests and clinical chemical tests", *Journal of Analytical Bio-Science*, **31** 307(2008)
- [2] Nagai Yutaka, Hiroshi Kondo, Yohko Kawai, "Summary of standardization in blood cell count using fresh blood", *Clinical Chemistry*, **38**, 408(2009)
- [3] Hiroshi Kondo, Yoko Sato, Nagai Yutaka, Noriyuki Tatsumi, "Current state of and measures for global standardization of blood cell analysis", *JJCLA*, **33**, 168(2008)
- [4] International Council for Standardization in Hematology; Recommendations for ethylenediaminetetraacetic acid anticoagulation of blood cell counting and sizing, *Am J Clin Pathol*, **100**, 371(1993)
- [5] International Council for Standardization in Hematology; Reference method for the enumeration of erythrocytes and leukocytes, *Clin Lab Haematol*, **16**, 131(1994)
- [6] International Council for Standardization in Hematology; Recommendations for "Surrogate reference" method for packed cell volume, *Lab Hematol*, **9**, 1(2003)
- [7] Hiroshi Kondo, Noriyuki Tatsumi, Kunihiro Kawabata, Makihiko Kuhara, "Evaluation of standard micro-hematocrit method NCCLS: H7-A", *Bulletin of Osaka College of Public Health*, **13**, 1993
- [8] International Council for Standardization in Haematology; Expert Panel on Hemoglobinometry: Recommendations for reference method for hemoglobinometry in human blood. *J Clin Pathol*, **49**, 271(1996)
- [9] International Council for Standardization in Hematology; Recommendation for the reference method for the packed cell volume. *Lab Hematol*, **7**, 148(2001)
- [10] International Council for Standardization in Hematology; Reference method for the enumeration of erythrocytes and leukocytes, *Clin Lab Haematol*, **16**, 131(1994)
- [11] International Council for Standardization in Hematology; The assignment of values to fresh blood used for calibrating automated blood cell counters. *Clin Lab Hematol*, **10**, 203(1998)
- [12] International Council for Standardization in Haematology; Expert Panel on Cytometry and International Society of Laboratory Hematology Task Force on Platelet Counting. Platelet counting by RBC/platelet ratio method. A reference method. *Am J Clin Pathol*, **115**, 460(2001)
- [13] Clinical and Laboratory Standards Institute; Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry, and Supravital Dyes); Approved Guideline—Second Edition. Clinical and Laboratory Standards Institute, Document H44-A2, 2004
- [14] Clinical and Laboratory Standards Institute; Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition. Clinical and Laboratory Standards Institute, Document H20-A2, 2007
- [15] Hiroshi Kondo, Masaaki Sugiyama, Atsushi Shirakami, Yohko Kawai, "External quality control of hematology testing using fresh blood". *Journal of Clinical Laboratory Medicine*, **58**, 621(2014)
- [16] Takayuki Takubo, Hiroshi Kondo, Naotake Sato, et. al., "Blood cell counts (reticulocytes) using reference blood cell analyzers of six manufacturers and 2007 external quality evaluation of white blood cell differentiation", *Journal of the Japanese Society for Laboratory Hematology*, **14**, 282(2013)
- [17] Toshiyuki Ikemoto, Takayuki Takubo, "Automated blood cell counters: measurement principles and considerations", *Kensa to Gijutsu*, **35**, 523(2007)
- [18] Hiroshi Kondo, Shinichiro Watanabe, Yoko Kawai, "Analysis of current state of external quality evaluation of automated blood cell analysis parameters using fresh blood", *Journal of the Japanese Society for Laboratory Hematology*, **13**, 189(2012)