

## **Reference measurement procedure for the enumeration of erythrocytes and leukocytes**

**Revision 01-03 2022-05-19**  
**Blood Cell Counting Standardization Subcommittee (BCC-SSC)**  
**The Japanese Society for Laboratory Hematology (JSLH)**

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## 1. Title

Reference measurement procedure for the enumeration of erythrocytes and leukocytes

## 2. Introduction

This document presents requirements for manufacturers of IVD Medical Devices (MDs) and Reference Laboratories located in Japan in documenting the calibration hierarchy for a measured quantity in human samples using a specified IVD MD. The document includes various model calibration hierarchies offering potential technical solutions for different kinds of measurands in establishing metrological traceability of assigned values for human samples and human calibrators based on The International Council for Standardization in Haematology (ICSH) documents.

The ICSH have defined a reference method for the enumeration of erythrocytes and leukocytes in published a guideline for the assignment of values to fresh blood used for calibrating automated blood cell counters in 1988 <sup>[01]</sup>. Then, ICSH published a reference method that extended ICSH1988 to include more accurate coincidence corrections for the enumeration of erythrocytes and leukocytes <sup>[02]</sup>. Since the previously published ICSH guidelines in 1994, hematology analyzers have evolved greatly. Almost manufacturers of automated hematology analyzer are used as higher order reference measurement procedure (RMP) according to 'In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples -' by the International Organization for Standardization (ISO)17511 <sup>[03]</sup>. The ICSH guidelines should be append some descriptions to comply with 'In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Requirements for content and presentation of reference measurement procedures (RMP)' by ISO15193 <sup>[04]</sup>. This document specifies technical requirements and documentation necessary to establish metrological traceability of values assigned to human samples and human calibrator for quantities measured by IVD MDs.

## 3. Scope

This document is described higher order RMP based on the ICSH guidelines for conforming ISO15193/17511, concerning of the assignment of values to fresh blood for enumeration of RBC and WBC. The reference method is provided reference measurement procedures to be comparable and ultimately metrologically traceable to measurement units and/or measurement standards and/or measurement

procedures of the highest metrological level.

Higher order RMP can be used for the following:

- a) in assessing performance properties of measuring systems comprising measuring instruments, auxiliary equipment as well as reagents,
- b) in demonstrating if there is a functional interchangeability of different routine measurement procedures purporting to measure the same quantity,
- c) in assigning quantity values to fresh blood as reference materials (calibrators) that are then used for purposes of calibration or trueness control of routine measurement procedures in manufacturer.

This document is applicable to:

- a) Objectives of measurement:

The assignment of values to blood used for calibrating automated hematology analyzer (automated blood cell counters) at reference laboratories of manufacturers.

- b) Types of sample material and interfering components for fresh blood calibrators:  
Healthy human fresh blood without some interfering components to RBC counting and WBC counting including of lysing process.

- c) Measurement interval:

Use analytical measurement interval covered with reference interval and clinical allowable interval.

- d) Measured volume interval:

Use clinical allowable upper limit and lower limit of volume axis for determining erythrocytes and leukocytes

- e) Calibration of volumetric devices:

Internationally standardized measurements of mass and traceable to National Institute of Standards and Technology (NIST) certification or other equivalent valid standard of accuracy.

- f) Nomenclature: ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. Int. J. Lab. Hematol. 2015, 37, 287–303 <sup>[05]</sup>

- g) Unit: ICSH standardization in haematology. Recommendation for standardization of haematology reporting units used in the extended blood count. Int J Lab Hematol. 2016 Oct;38(5):472-82 <sup>[06]</sup>.

This document is not applicable to:

- a) Stored blood calibrators and trueness control materials for IVD MDs
- b) Control materials that are used only for internal quality control purposes to assess the imprecision of an IVD MD

The scope of this document is NOT to propose the different RMP from the one published by ICSH but to append the contents which are required by ISO 17511 and 15193 as described in Introduction. Except for the appended description, the other contents follow the reference method by ICSH.

#### **4. Warning and safety precautions**

Warnings and safety precautions regarding blood sample handling, transportation of infectious substances and determining the safety level depending on whether it contains bloodborne pathogens should be based on the policies described in the following documents for laboratory biosafety level.

- a) Laboratory biosafety manual 4th ed. 2020.12 <sup>[07]</sup>
- b) Guidance on regulations for the transport of infectious substances 2021-2022. applicable as from 1 January 2021 <sup>[08]</sup>
- c) OSHA's Bloodborne Pathogens Standard (29 CFR 1910.1030) <sup>[09]</sup>

#### **5. Measurement principle and method**

The chamber counting is replaced by a single channel semi-automated electronic counter using the aperture-impedance principle <sup>[10]</sup> provided that a known volume of diluted blood is displaced through the orifice. Only semi-automated single channel aperture-impedance particle counters are available. The instrument should have an impedance cell counter with optimized orifice diameter and length of the diameter, detector for displaced volume through the orifice and coincidence correction function. Measurement principles and method is shown to the followings <sup>[11]</sup>:

##### **- Cell counter**

Cell counts should be performed using a semi-automated single channel counter of design specifications which permit counting, by electronic means, of all the cells in a known displaced volume of the diluted blood sample. Whilst aperture-impedance counters could be used for this purpose. The design of the electronic cell counting instrument should be such that each cell is counted once and only once. The probability of re-circulation of particles through the sensing zone must be low and any impulses so created should be below the appropriate lower threshold. The detection electronics should have the ability

to discriminate, with a low probability of error, by pulse amplitude between the cells of interest, other cells and electrical noise. The detection electronics should have sufficient signal to noise ratio to identify erythrocytes and leukocytes and a signal amplitude roughly proportional to cell volume. The lower signal threshold should be adjusted to an incremental accuracy equivalent to  $\pm 5$  fL cell volume or less over the range 0-100 fL. The following considerations should be made to ensure that the effect on erythrocyte and leukocyte counts is less than or equal to 1.5% for erythrocyte count and 2.0% for leukocyte count.

- a) The volume measurement range should include the entire volume range of erythrocytes<sup>[12]</sup> and leukocytes<sup>[10] [13]</sup>. Note: The volume range of leukocytes may vary depending on the hemolytic agent used.
- b) The lower volume limit of erythrocytes should be such that if the upper volume limit of platelets overlaps, measures should be implemented to automatically control the lower threshold of erythrocytes to minimize the effect of this overlap.
- c) Since the upper volume limit of erythrocytes slightly overlaps with the lower volume limit of leukocytes, measures should be implemented as necessary, such as setting the upper threshold of erythrocytes to minimize the effect of this overlap.
- d) The lower volume limit of the leukocyte measurement should be set so that measures are taken as necessary to ensure that the lower volume limit is not affected by erythrocyte ghost (debris) caused by hemolysis. For example, using a lower threshold for the large debris detection which is set at a volume smaller than the lower volume threshold for leukocytes, the difference in counts by both thresholds may be used to detect the effect of debris.

The design should be such that the probability of recirculation of cells inside the orifice tube is low. Cavitation (trapped air bubbles) and turbulence must be avoided. The sample flow lines must not change volume in response to changes in fluid pressure nor should their materials contribute any measurable cell loss due to adherence.

- **Coincidence correction:**

Coincidence correction may be provided automatically or by means of a coincidence correction chart. The counting error after coincidence must not



exceed 2%. The total electronic dead time for a signal pulse must not affect the coincidence correction.

- **Displaced liquid volume through the orifice:**

The liquid volume displaced during the counting period must be known to within an accuracy of 1% traceable to a national or international metrological standard. This displacement can be achieved by the motion of incompressible fluid column (mercury or distilled water other suitable means) such as "manometer" built-in reference counter. The differential displacement like thermal effects or other affecting elements must be considered.

A quantification equipment for volume of displaced liquid through the orifice connects a manometer in reference counter to the quantification equipment and has the capability to measure the weight of a moving liquid column in the manometer and convert it to a moving volume. The flow path between the manometer and the equipment should be filled with incompressible liquid and measures should be taken to prevent bubbles from entering the flow path. In Use of the quantification equipment, the calibration procedure and evidence for volume measurement shall be validated. There is an example of using mercury as the liquid column. The calibration of mercury manometer volume is shown in Appendix A <sup>[14]</sup>. Volumetric quantification using the water column can also be implemented to solve environmental issues. For quantitative validation by water column weight, the following measures should be taken in consideration of the main error factors.

- a) Metering syringe: Use glass micro syringes or equivalent to ensure accuracy. The movement of the metering syringe should be automated so that it can aspirate or dispense the volume of the column of liquid to be measured at a constant speed. The speed of movement of the column of liquid should be set the speed to the speed set by the fluid control of the reference counter.
- b) The amount of fluid remaining on the inner wall in the manometer of the reference counter: It is advisable to check whether the error factor is significant by checking the difference in the amount of water column movement when the moving speed of the liquid column is set as low as possible and when the moving speed of the reference counter is set.

- c) Weighing equipment: Select equipment that can measure 4 to 5 significant digits (equivalent to 0.1 to 0.01  $\mu\text{L}$ ), which is necessary to ensure valid quantitative accuracy.
- d) Weighing environment: Take measures to prevent the effects of static electricity, vibration, and wind.
- e) Room temperature control: Set the temperature at 20°C, which is the standard temperature for glass weighing instruments, and take measures to correct the capacity according to the measured temperature.
- f) Weighing bottle: To minimize the effect of evaporation of the metered amount of water, use a weighing bottle with a lid or take other measures.
- g) Sampling outlet: To minimize the volume of dispensed droplets and to minimize the amount of a droplet remaining on the outside of the needle tip, measures such as using an LC tip with a 90° cut at the tip of the needle should be taken.
- h) Sampling: Conduct sampling as close as possible to the weighing environment equipment.

## **6. Apparatus**

The RMP requires the dedicated analyzer and some equipment.

### **6.1. Name of the apparatus**

Single-channel semi-automated aperture-impedance cell counter

### **6.2. Essential performance properties**

The apparatus for the RMP should fulfill the properties below as the essential performance properties. The basic performance characteristics described in “16. Analytical reliability” should be validated by manufactures.

- Probability of re-circulation of particles through the sensing zone must be low and any impulses so created should be below the appropriate lower threshold.
- Detection electronics should have the ability to discriminate, with a low probability of error, by pulse amplitude between the cells and interest, other cells and electrical noise.
- Instrument should have an orifice diameter 80-100  $\mu\text{m}$  and length 70-100 [02]. If the instrument has the other orifice diameter and length, it should be confirmed

that the instrument has the same level of analytical performance defined in this document.

- Volume displaced during the counting period must be known to within an accuracy of 1% traceable to a national or international metrological standard.
- This displacement can be achieved by the motion of a mercury column or other suitable means.
- The differential displacement due to thermal effects should not be greater than 0.1% per °C.
- Noise must be below the threshold level. Sufficiently low cell counts when measuring background. To avoid artefactual signals, the threshold settings should ensure to sufficient level not to be affected noise. The cell counting number in blank measurement should be confirmed low enough not to affect the counting.

### 6.3. Other equipment

To prepare the appropriate equipment described below is essential to assure the results of the RMP with enough quality.

- **Pipettes**

Positive displacement pipettes should be used, calibrated to an accuracy of  $\pm 0.5\%$ , validated by a process traceable to a primary metrological standard.

- **Volumetric flasks**

Grade A volumetric flasks made of borosilicate glass, should be used, each with a stated volume which has been tested by an appropriate national standards body, or which has been checked by the individual user by means of a gravimetric method using certified weights and corrections for buoyance and temperature.

- **Counting vials**

The counting vial should have a minimum volume of 10 ml. It should be of sufficient height that the entry orifice of the electronic cell counter is at approximately half the depth of the fluid before counting commences and there should be more than 1 cm of fluid above that orifice after the completion of counting. Before use, the vial should be cleaned free of chemical contaminants and adventitious particles. It is necessary to confirm that cells do not adhere to the counting vial and cause a progressive fall in the count. This is ascertained by testing a number of vials from each batch. Counts are made at measured

intervals after the dilutions have been placed in the counting vials. The dilution should be mixed in the vial before counting.

## **7. Reagents**

The reagents for the RMP, it should have the appropriate quality defined by manufactures and the operator should follow the any description such as storage conditions, shelf life, and so on described on the labels or in the package inserts.

### **7.1. Diluent**

The diluent must be a sterile, non-toxic, buffered salt solution suitable for the measurement system. The diluent must not cause cell lysis or later cell volume during diluting blood to the completion of measurement; hence it must be verified that there is no significant volume change that may affect cell count. Its tonicity and the range should be stated. The constituents and their concentrations must be given on the label of the container or on the package insert. When counted in the vial, it must contain less than  $1 \times 10^5$  particles per liter measurable at the threshold setting for the cells which are to be counted. The operator should follow the any description such as storage conditions, shelf life, and so on described on the labels or in the package inserts.

### **7.2. Lytic Agent**

The lytic agent is necessary when white blood cells are to be counted in a whole blood sample and the red cells must be first be lysed. The lytic agent must not affect the ability of the white cells to be counted whilst the red cell stroma must be reduced to a residue which does not contribute to the white cell count. For example, typical traditional hemolytic agents used since the early days include quaternary ammonium salts, anionic surfactants, cationic surfactants or and non-ionic surfactant. The different hemolytic agents may change the volumetric range of leukocytes. The operator should follow the any description such as storage conditions, shelf life, and so on described on the labels or in the package inserts.

### **7.3. In-house reagents**

If the RMP requires the procedure to prepare the reagents, the operator should prepare the dedicated SOP describing the procedure.

#### **7.4. Expression of concentration**

Manufacturers should prepare the document describing the concentration expressed as amount-of-substance concentration of the necessary reagents for the RMP. The operator should prepare the document describing the concentration for the in-house reagents.

### **8. Sampling and sample**

Fresh venous blood specimens should be obtained by syringe or evacuated container. Ideally the period between venous sampling and processing should not exceed 8h of venesection <sup>[15]</sup>.

#### **8.1. Tubes**

Standard evacuated containers, 10 – 12×75mm, containing 5ml blood require an air bubble comprising at least 20% of the tube volume.

#### **8.2. Blood collection**

Specimens should be anticoagulated with K2 EDTA in a final concentration of 3.7 – 5.4 µmol (1.5 – 2.2mg/ml) and placed in containers with allow sufficient air remaining to enable proper mixing <sup>[16]</sup>.

#### **8.3. Storage of the specimen**

Specimens should be maintained at  $20 \pm 2^{\circ}\text{C}$  until processing. Ideally the period between venous sampling and processing should not exceed 8h.

#### **8.4. Sample handling**

Before testing the specimen should be well mixed by gently inverting the container. The number of inversions of a container, to achieve adequate homogeneity, will depend on the type and dimension of the container. Blood samples should be processed within 4-8 h of venesection <sup>[15]</sup>.

#### **8.5. Special notes**

- Liquid anticoagulants are not recommended because the cell count is affected due to the dilution by the anticoagulants.
- Vortex mixers should not be used.

- Non-standard tubes, particularly narrower tubes require more complete inversions.
- Specimens should be rejected if there is visible haemolysis or if micro-clots are present.

## **9. Control materials and blank materials**

It is recommended for the manufactures of the RMP to provide the appropriate control materials so that the quality of the RMP in each laboratory can be assured by performing the internal quality control.

### **9.1. Control materials**

Commercial control materials are available to perform quality control for the measurement system. Pre-analytical procedure should be performed following the description of the package insert for the materials.

### **9.2. Blank materials**

As described in 7.1., the diluent for the measurement system must contain less than  $1 \times 10^5$  particles per liter measurable at the threshold setting for the cells which are to be counted, therefore the diluent is available as the blank materials. The other solutions which can be assured it has same quality with diluent are also available as the blank materials.

## 10. Analytical solution

Coincidence correction should be performed for every blood specimen which is to be counted. Make two primary dilutions (each of 0.1 ml blood plus 20 ml diluent). Take 0.02, 0.04, 0.06 and 0.08 ml of the primary dilutions and add these to 20 ml aliquots of diluent to make two sets of four secondary dilutions, the most concentrated of these, i.e., 0.08 + 20 ml, having a dilution factor of 1/50451. Each secondary dilution is then counted as follows:

| Secondary dilutions | 0.02+20 | 0.04+20 | 0.06+20 | 0.08+20 |
|---------------------|---------|---------|---------|---------|
| No. of counts       | 12      | 6       | 4       | 3       |

The diluted sample must be transferred to counting vials and gently mixed for about 30 s to get rid of bubbles. The count must be completed within 5 min of performing the dilution.

## 11. Preparation of the apparatus

### 11.1. Requirement for the manufactures

For manufactures providing the measurement system, it is required to prepare the appropriate instruction for the users which covers the items such as warning and safety precautions, assembly, checking that the tolerance limits of performance quantities are not exceeded, operating mode and user's preventive maintenance.

### 11.2. Requirement for the laboratories

Laboratories using the apparatus for the RMP, the operator should follow any description described in the instruction.

## 12. Operation

Regarding the operation including the sample measurement, the operator should follow the defined measurement steps and should notice the errors which would happen during the operation. Exclude certain observations from data analysis if there is an assignable cause. Assignable causes are defined as events in the following categories:

- a) Failure to adhere to study-specific protocol or procedures
- b) Instrument failure
- c) Operator-related (e.g., removal of sample before complete aspiration occurs)
- d) Specimen-related (e.g., hemolyzed or clotted)
- e) Any fresh blood numerical result that has data-invalidating flags as described in the operating instructions for each of the instruments

Exclude only the invalidated numerical results for a given specimen run. All other valid numerical results for other measurands within that run may be used for analysis, provided that such measurands are not potentially analytically impacted by other invalidated results, as defined by the manufacturer.

### 12.1. Sequence of measuring steps

- 1) Sample preparation following the description in 8. Sampling and sample and 10. Analytical solution.
- 2) The apparatus should be prepared following the description in 11. Preparation of the apparatus
- 3) Measurements are made 3 times with the 100% sample, 4 times with the 75% sample, 6 times with the 50% sample, and 12 times with the 25% sample using the blood cell counter, and the counts of each sample strength are summated.
- 4) Draw the graph based on the measurement results and calculate the erythrocyte/leukocyte count following the description in 13. Data processing

### 12.2. Errors

The errors below should be cared during the operation and the error which happens during the operation should be recorded in the report.

#### - Analysis of error

The maximum permissible bias is 2.0% for red cell counts and 4.0% for white cell counts. Potential sources of error are listed below and apply to both red and white cell counting.

#### - Sampling error



This error arises if the measured sample is not representative of the original whole blood specimen. Factors to be considered include poor mixing, haemorheological effects in extracting the sample and the inaccuracy in dilution.

- **Transport error**

Errors due to transport occur if each and every cell is not counted in a known volume of diluted blood. Such errors are caused by sedimentation, cell loss and/or recirculation, as well as the inaccuracy of the displaced volume.

- **Counting error**

Errors can arise if the final count is distorted because of improper discrimination, spurious counts or inaccurate correction for coincidence loss. The imprecision of the reference cell count should be no greater than 1%. Potential sources of error include extracting the sample, dilution, the imprecision of the displaced volume and the statistical limitation of the number of cells counted.

### **13. Data Processing**

The calculation of measurement results shall contain the following steps;

- Processing of initial data
- Construction of measuring function
- Minimum number of points to generate the measuring function
- Kind-of-quality & measurement unit
- Model for statistical treatment of measured quantity values
- Number of replicates measured quantity values necessary to calculate a measurement result, their allowable maximum difference and the equation used
- Number of significant figures in the measurement result and any rounding procedure
- Calculation of measurement uncertainty

#### **13.1 Calculation of measurement results**

**a) Processing of initial data:**

Initial data are “measured count of 50,000-fold dilution sample”.

Initial data shall be corrected by coincidence-correction study.

**b) Construction of measuring function:**

RBC count can be obtained by the following formula (1) and (2);

X: Blood Cell Count in WB sample ( / L)  
 $c_c$ : Corrected Count of primary dilution (50,00-fold) sample  
 $v_M$ : Displaced volume by the manometer (L)  
 $v_D$ : Displaced volume by the micro-dispenser (L)  
 $v_F$ : Nominal Volume of the volumetric flask (L)  
  
a : y-axis of linear-regression formula for coincidence-correction  
  
b : Slope of linear-regression formula for coincidence-correction  
 $c_m$ : Observed Count of (L) of primary dilution (50,00-fold) sample

$$X = \frac{c_c}{v_M} \times \frac{v_D + v_F}{v_D} \quad (1),$$

$$c_c = a + bc_m \quad (2)$$

**c) Kind-of-quantity & measurement unit:**

Kind-of-quantity is RBC concentration, erythrocyte count per 1 L of whole blood sample. Measurement unit for RBC and WBC is “ x 10<sup>12</sup> / L”, and “ x 10<sup>9</sup> / L”, respectively.

**d) Model for statistical treatment of measured quantity values:**

Statistical model shall be given in the following formula (1) and (3);

$$X = \frac{c_c}{v_M} \times \frac{v_D + v_F}{v_D} \quad (1),$$

$$c_c = a + bc_m + \varepsilon_{LR} + \delta_{CA} \quad (3)$$

X: Blood Cell Count in WB sample ( / L)  
 $c_c$ : Corrected Count of primary dilution (50,00-fold) sample  
 $v_M$ : Displaced volume by the manometer (L)  
 $v_D$ : Displaced volume by the micro-dispenser (L)  
 $v_F$ : Nominal Volume of the volumetric flask (L)  
  
a : y-axis of linear-regression formula for coincidence-correction  
  
b : Slope of linear-regression formula for coincidence-correction  
 $c_m$ : Observed Count of (L) of primary dilution (50,00-fold) sample  
 $\varepsilon_{LR}$ : Deviation of corrected-count originated by linear regression  
 $\delta_{LR}$ : Deviation of corrected-count originated by volume of equipment used for dilution-line preparation

**e) Description of any algorithm used:**

There are possibly 2 options for assigning the RBC count – ICSH original recommended procedure and its modified procedure, as follows;

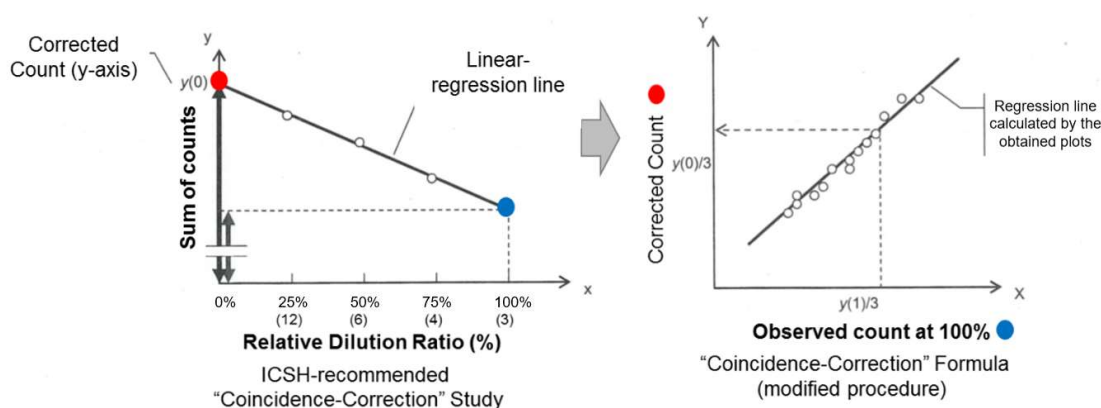
ICSH original RMP

Coincidence-correction study should be performed, sample-by-sample.

Modified Procedure

“Coincidence-Correction Formula” can be applied, which shall be created by the validation study based on multiple samples & analyzers.

\* As the ICSH original RMP requires more time to do, the modified procedure can be used as an optional RMP.



**f) Number of replicates measured quantity values necessary to calculate a measurement result, their allowable maximum difference and the equation used:**

|                  | 25% | 50% | 75% | 100% |
|------------------|-----|-----|-----|------|
| # of measurement | 12  | 6   | 4   | 3    |

**g) Number of significant figures in the measurement result and any rounding procedure;**

RBC count and WBC count assigned by RMP are rounded to two places of decimals; e.g. RBC: 4.965 -> 4.97 ( $\times 10^{12} / L$ ), WBC: 7.532 -> 7.53 ( $\times 10^9 / L$ ).

**h) Calculation of measurement uncertainty**

See the budget sheet in Appendix C.

### **13.2 Comparison with measurement results obtained by other measurement procedures**

If relevant for comparability, comparative data shall be given on measurement results on various types of sample to which the reference measurement procedures is claimed to apply with the procedure presented and with alternative measurement procedures differing in measurement principle, measurement method or details of measurement procedure, such as national metrology institute: PTB's reference method, AIST's optical particle count method using monodisperse polystyrene latex (PSL) standard solution<sup>[17]</sup>, etc.

PTB: Physikalisch-Technische Bundesanstalt National Metrology Institute

AIST: The National Institute of Advanced Industrial Science and Technology

## **14. Reporting**

The report of the results should contain the information described below.

- Identification of source and type of sample
- Date of sampling and possibly date of measurement
- Results with measured quantity name, numerical value, and measurement unit
- Statement of measurement uncertainty
- Observations of unusual properties of sample
- Observations as regards unusual features of the measurement procedure or use of modifications
- Physiological and clinical information, if relevant

## **15. Validation of a reference measurement procedure**

The reference laboratory shall validate the reference measurement procedure that it is fit for its intended use. The validation may include the following items;

- Comparison of measurement results achieved with other measurement procedures
- Inter-laboratory comparisons
- Performance validation using reference materials
- Systematic assessment of factors influencing the results
- Assessment of the measurement uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience

### **15.1 Comparison of measurement results achieved with other measurement procedures**

Using multiple fresh whole blood samples (at least 50 samples), the comparison study

as a validation for secondary standards (hematology analyzers) of RBC count and WBC count shall be performed. The samples used for this comparison study shall have – RBC:  $3.0 - 6.5 ( \times 10^{12} / L )$  and WBC:  $3.0 - 10.0 ( \times 10^9 / L )$ . Do correlation analysis between the both methods, and confirm if the  $R^2$  (coefficient determination) is  $> 0.95$  for RBC and WBC.

## **15.2 Inter-laboratory comparisons**

Perform the inter-laboratory comparisons among the reference laboratories performing the reference measurement procedures for RBC and WBC, periodically. If it is not available, the alternative methods are participating in the External Quality Assessment or proficiency testing program, such as CAP, UK-NEQAS and INSTAND. The recommended frequency of inter-laboratory comparisons is more than twice per year, for monitoring the z-score (SDI) or %bias to the target value.

## **15.3 Performance validation using reference materials**

Though there is no certified reference materials for blood cell counting, one possible way to validate the performance of reference counter is to use the calibration standards for number concentration of liquid-borne particles – e.g. PSL standard solution which is provided and value-assigned by the national metrological institute.

## **15.4 Systematic assessment of factors influencing the results**

n/a

## **15.5 Assessment of the measurement uncertainty of the results**

Combined standard measurement uncertainty of assigned values for these kinds of reference materials for hematology analyzers (that are not calibrators) shall be estimated by the manufacturer and provided to end users on request. The calibration hierarchy is able to be clarified by using measurands defined by international harmonization protocol (No CRM; not traceable to SI) in ISO17511-2020 (5.6 Cases with metrological traceability supported by an international harmonization), and more detailed explanations can be found at ISO21151-2020<sup>[18]</sup>. The model of calibration hierarchy for metrological traceability is shown in Appendix B. The assessment of the measurement uncertainty of RBC and WBC count performed by the reference measurement procedure shall be evaluated and documented in the reference laboratory according to the JCGM 200:2012<sup>[19]</sup>.

## 16. Analytical reliability

The reference laboratory shall demonstrate the analytical reliability of the reference measurement procedure for RBC and WBC, for the following items;

- Linearity
- Analytical influence quantities
- Blank measurement
- Measurement uncertainty
- Measurement precision
- Repeatability
- Intermediated precision
- Reproducibility
- Detection limit
- Lower and higher measurement limits

### 16.1 Linearity

The linearity range of the reference measurement procedure for RBC and WBC shall be stated and demonstrated, which should cover the measuring range of the whole blood samples – RBC:  $3.0 - 6.5 ( \times 10^{12} / L )$  and WBC:  $3.0 - 10.0 ( \times 10^9 / L )$ .

### 16.2 Analytical influence quantities

The analytical influence quantities in the whole blood samples used for the reference measurement procedure for RBC and WBC shall be stated and demonstrated, such as visible hemolysis, micro-clots or turbidity.

### 16.3 Blank measurement

Before measuring the diluted whole blood samples, confirm if the blank measurement count is lower the criteria – e.g. criteria for blank measurement  $< 50$ ;  $< 0.2\%$  for RBC,  $< 0.5\%$  for WBC.

### 16.4 Measurement uncertainty

The measurement uncertainty of the reference measurement procedures for RBC and WBC shall be evaluated and stated in the Uncertainty budget sheet (see Appendix C).

There are the following steps to evaluate the measurement uncertainty <sup>[11]</sup>;

- List up the major uncertainty sources such as listed in Appendix C.
- Estimate standard uncertainty for major uncertainty source (type A, type B) :  $u_i$
- Calculate combined standard uncertainty:  $u_c$

- Determine expanded standard uncertainty:  $U = k u_c$  (coverage factor,  $k=2$ )
- State the measurement uncertainty :  $y \pm U$

Prepare documentation for evaluating process of the measurement uncertainty to show the analytical reliability. The model equation for measurement and equation of evaluating uncertainty is shown in Appendix D which describes more detailed model than the model in Appendix B <sup>[14]</sup>. The manufactures shall evaluate the measurement uncertainty of the reference measurement procedures for RBC and WBC following these models.

### 16.5 Measurement precision

The measurement precision shall be evaluated for the following conditions;

- Repeatability conditions, i.e. the intra-run situation
- Intermediate precision conditions, i.e. defined between-run situation in a given laboratory
- Reproducibility conditions where several laboratories are involved

#### a) Repeatability

Prepare the diluted samples of whole blood or QC samples for RBC and WBC, and run 10 times consecutively. Calculate the mean, standard deviation (SD) and coefficient of variance (%CV) for RBC and WBC.

#### b) Intermediate precision (Internal quality control)

Perform the QC using the stabilized QC materials – e.g. 4-runs per day x 25 days (2-3 time / week). Calculate the mean, SD and %CV for RBC and WBC.

#### c) Reproducibility (Interlaboratory comparisons)

Perform the QC using the stabilized QC materials at multiple reference laboratories – e.g., 4-runs per day x 45 days (2-3 time / week) at 3 reference laboratories. Do ANOVA analysis and calculate the means, inter-lab and intra-lab SDs and %CVs for RBC and WBC.

### 16.6 Detection limit

n/a

### 16.7 Lower and higher measurement range

The lower and higher measurement range shall be demonstrated in the linearity study results for RBC and WBC.

## **17. Quality assurance**

Laboratories should perform the internal quality control before the sample measurement and perform the interlaboratory comparisons to assure the quality of the RMP in each laboratory,

### **17.1. Internal quality control**

- Internal quality control should be performed prior to the sample measurement.
- Appropriate control material which has the assay value for RBC and WBC should be selected. It is preferable to use 2 levels of control materials.
- QC chart with target and limit values should be prepared based on the appropriate statistical calculation in each laboratory.
- The operator should strictly follow the pre-analytical procedure described in the package inserts for the materials.
- Measure for each control material at least once before and after sample measurement and plot the result in the QC chart. Outlier should be checked measurement value for each control before sample measurement.
- The example of Internal quality control is shown in Appendix E.

### **17.2. Interlaboratory comparisons**

- Interlaboratory comparisons should be planned and performed regularly (e.g. once a year, once per 3 years)
- Fresh human blood collected by the appropriate procedure (it is preferable to prepare the standard operation procedure about fresh human blood collection for interlaboratory comparison) or commercial control materials are available for interlaboratory comparison.
- Organizer of interlaboratory comparisons should prepare the instruction covering the pre-analytical and measurement procedure.
- Organizer should calculate the statistics based on the data submitted from laboratories and define the outlier based on the defined criteria of the comparison.
- For the outlier laboratory, the operator in the laboratory should look for the cause, perform the countermeasure, and record the measure.
- The example of interlaboratory comparison is shown in Appendix F.



## **18. Special cases**

The RMP is described following the method established in 1994. The laboratory can partially customize the method as the special cases. In case applying such special cases, the laboratory should assure that the compatibility of the results with the original method.

### **18.1. Reduced coincidence correction method**

Coincidence correction should be performed for every blood specimen which is to be counted, however in case to measure many specimens, it is available to perform the coincidence correction by applying the regression line obtained by the measurement for some samples.

## References

- [01] The assignment of values to fresh blood used for calibrating automated blood cell counters. Clin Lab Haematol 1988; 10: 203-212
- [02] Reference method for the enumeration of erythrocytes and leucocytes. Prepared by the Expert Panel on Cytometry. Clin Lab Haematol 1994; 16: 131–138.
- [03] In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples – ‘by the International Organization for Standardization (ISO)17511
- [04] In vitro diagnostic medical devices – Measurement of quantities in samples of biological origin – Requirements for content and presentation of reference measurement procedures (RMP)’ ISO15193 .
- [05] ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. Int. J. Lab. Hematol. 2015, 37, 287–303.
- [06] ICSH standardization in haematology. Recommendation for standardization of haematology reporting units used in the extended blood count. Int J Lab Hematol. 2016 Oct;38(5):472-82
- [07] Laboratory biosafety manual 4th ed. 2020.12
- [08] Guidance on regulations for the transport of infectious substances 2021-2022. applicable as from 1 January 2021
- [09] OSHA's Bloodborne Pathogens Standard (29 CFR 1910.1030)
- [10] Davis B, Barnes PW. Chapter 3, Automated Cell Analysis: Principles, In: Laboratory Hematology practice, 1st edn. International Society for Laboratory Hematology (Kottke-Marchant K, Davis B eds). New Jersey: John Wiley & Sons; 2012.
- [11] Shinkai E, Shirakami A, Fujimoto K, The reference measurement procedure for erythrocyte enumeration. Kensa to Gijutsu, 2008; Vol. 36(6): 518-522
- \*English version: Sysmex Int 2009; 19:1-5 (This article was translated and republished with permission from IGALU-SHOIN Ltd.)

[12] Constantino BT. The Red Cell Histogram and The Dimorphic Red Cell Population. Laboratory Medicine. 2011 May; 42(5): 300-308.

[13] Niitani K. Advance on blood cell counting, especially on volumetric analysis and related problems. Jpn J Clin Hematol. 1984. 25(6). 768-775

[14] E.Shinkai, A.Shirakami, K.Fujimoto; Research Group for Case Studies on Uncertainty Evaluation, NMIJ (National Metrology Institute of Japan) Uncertainty Club, 183-195 (2009)

[15] International Council for Standardization in Haematology, Writing Group, Briggs C, Culp N, Davis B, d'Onofrio G, Zini G, Machin SJ; International Council for Standardization of Haematology. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. Int J Lab Hematol. 2014 Dec;36(6):613-27. PMID: 24666725

[16] Recommendations of the ICSH for EDTA Anticoagulation of blood for blood counting and sizing, A.J.C.P October 1993

[17] Y. Kuruma et.al, A. Validation of number concentration measurement equivalent to ICSH reference method for impedance-type blood cell counter by comparison with a primary standard for number concentration of liquid-borne particles, XXXIII International Symposium on Technical Innovations in Laboratory Hematology, Melbourne, Australia - Melbourne Convention and Exhibition Centre, May 21-23, 2020

[18] ISO21151-2020 "In vitro diagnostic medical devices — Requirements for international harmonisation protocols establishing metrological traceability of values assigned to calibrators and human samples"

[19] JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM) 3rd edition, 2008 version with minor corrections

## Appendices

### Appendix A. Example of calibration of mercury manometer volume

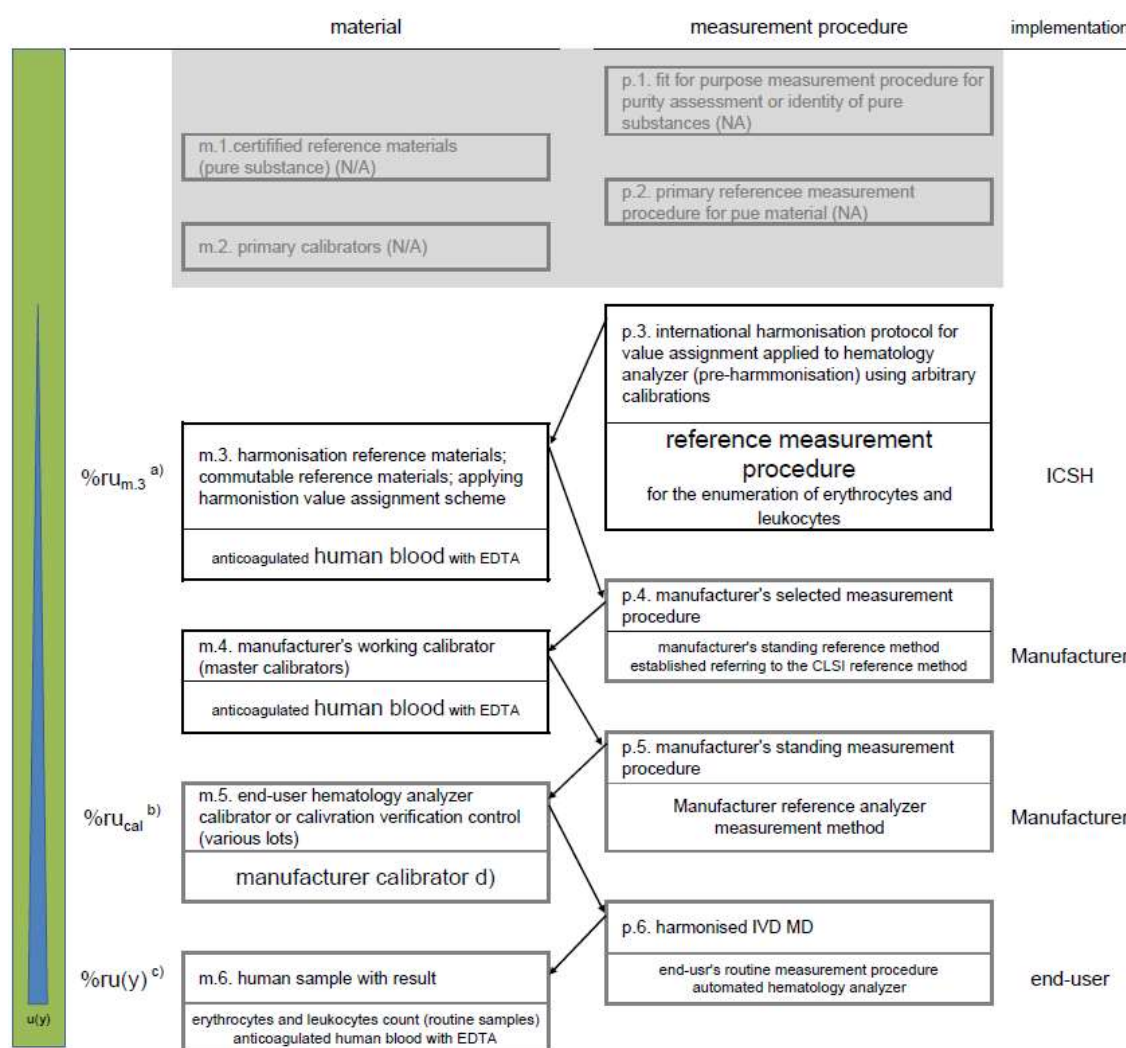
Before equipping a manometer with the reference counter, the manometer volume can be calibrated using a dedicated quantitative device consisting of quartz-glass passage and special syringe to quantify the mass of mercury column's displacement, which was precisely calibrated by the metrological institute, JCSS (Japan Calibration Service System)<sup>[14]</sup>. An example of calibrating the mercury manometer volume is shown below.

|                                   | Measured Volume ( mL ) |          |          |
|-----------------------------------|------------------------|----------|----------|
| 1                                 | 1.0496                 |          |          |
| 2                                 | 1.0497                 |          |          |
| 3                                 | 1.0501                 |          |          |
| 4                                 | 1.0504                 |          |          |
| 5                                 | 1.0502                 |          |          |
| Mean                              | 1.05000                |          |          |
| SD                                | 0.00034                | Criteria | Judgment |
| CV (%)                            | 0.032%                 | < 0.5%   | OK       |
| Expanded Uncertainty<br>( $k=2$ ) | 0.00221                | < 1%     | OK       |

|                       |         |
|-----------------------|---------|
| Certified Volume (mL) | 1.05000 |
|-----------------------|---------|

## Appendix B. Model of calibration hierarchy for metrological traceability

The model of the calibration hierarchy in the enumeration of erythrocytes and leukocytes shall be selected cases with metrological traceability supported by an international harmonization protocol, as shown in the following figure. The hierarchy shall apply to cases in which the measurand in human samples is defined by an international harmonization protocol. There are no internationally agreed upon reference measurement procedures, no primary reference materials, no conventional reference measurement procedures or reference materials, and no metrological traceability to the SI.



Measurand defined by international harmonization protocol (No CRM; not traceable to SI).

Materials [m.1], [m.2], and measurement procedures [p.1] and [p.2] are not applicable (N/A)

( ISO 17511:2020, 5.6 Cases with metrological traceability supported by an international harmonization )

- a) Relative percent combined value assignment uncertainty of the [m.3] reference material, calculated according to the following formula:

$$\%ru_{m.3} = (\%ru_{c-p.1}^2 + \%ru_{RW-p.3}^2)^{(1/2)}$$

where

$\%ru_{c-p.1}$  is the relative percent combined standard measurement uncertainty for the [p.1] higher order

MPs with relation to e.g. thermometry, volumetry, time, length, etc.; N/A

$\%ru_{RW-p.3}$  is the relative percent standard deviation (CV%) for MP [p.3] under repeatability conditions.

- b) Relative percent combined value assignment uncertainty of hematology analyzer calibrator [m.5] calculated according to the following formula:

$$\%ru_{cal} = (\%ru_{m.3}^2 + \%ru_{RW-p.4}^2 + \%ru_{RW-p.5}^2)^{(1/2)}$$

where  $\%ru_{RW-p.4}$ ,  $\%ru_{RW-p.5}$ , represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

- c) Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user hematology analyzer, calculated per the following formula:

$$\%ru(y) = (\%ru_{cal}^2 + \%ru_{RW-p.6}^2)$$

where  $\%ru_{RW-p.6}$  is the relative percent standard uncertainty of hematology analyzer based on long-term precision (repeatability conditions of measurement).

- d) The documentation of the status of the material's commutability with human samples when used with any intended measurement procedures, including hematology analyzers: 123-456789 (manufacturer's report number)

## Appendix C. Uncertainty Budget of RMP for RBC enumeration

The measurement uncertainty of the reference measurement procedures for RBC and WBC shall be evaluated and stated in the Uncertainty budget sheet. There are the following steps to evaluate the measurement uncertainty <sup>[11]</sup>;

- List up the major uncertainty sources such as listed in Appendix C.
- Estimate standard uncertainty for major uncertainty source (type A, type B) :  $u_i$
- Calculate combined standard uncertainty:  $u_c$
- Determine expanded standard uncertainty:  $U = k u_c$  (coverage factor,  $k=2$ )
- State the measurement uncertainty :  $y \pm U$

Prepare documentation for evaluating process of the measurement uncertainty to show the analytical reliability.

| symbol   | Source of Uncertainty                  | 値 ±                             | Type | Probability Distribution           | Divisor    | Standard Uncertainty             | Sensitivity Factor                                      | Std. Uncertainty (measured unit) | Note   |
|----------|--|---------------------------------|------|------------------------------------|------------|----------------------------------|---|----------------------------------|--|
| $U_{R3}$ | Repeatability of RMP                   | 0.012<br>( $\times 10^{12}/L$ ) | A    | Normal Distribution                | 2          | 0.0058<br>( $\times 10^{12}/L$ ) | 1.0   | 0.0058<br>( $\times 10^{12}/L$ ) | Mean of RBC count by Reference Counter<br>5.072 ( $\times 10^{12}/L$ ) |
| $U_V$    | Calibration of Manometer Volume        | 2.6<br>$\mu L$                  | B    | Normal Distribution                | 2          | 1.3<br>$\mu L$                   | 0.005<br>( $\times 10^{15}/L^2$ )                       | 0.0062<br>( $\times 10^{12}/L$ ) | Calibration Results of Manometer # XX<br>1048.3 $\mu L$                |
| $U_{DC}$ | Calibration of Micro-dispensor Volume  | 0.11<br>$\mu L$                 | B    | Normal Distribution                | 2          | 0.054<br>$\mu L$                 | 0.26<br>( $\times 10^{15}/L^2$ )                        | 0.014<br>( $\times 10^{12}/L$ )  | Calibration Results of Micro-dispensor<br>19.86 $\mu L$                |
| $U_{MS}$ | Sampling by Micro-dispensor            | 0.046<br>$\mu L$                | A    | -                                  | 1          | 0.046<br>$\mu L$                 | 0.26<br>( $\times 10^{15}/L^2$ )                        | 0.012<br>( $\times 10^{12}/L$ )  | Pooled Uncertainty<br>19.86 $\mu L$                                    |
| $U_F$    | Calibration of Volumetric Flask Volume | 0.40<br>mL                      | B    | Rectangular Distribution           | $\sqrt{3}$ | 0.23<br>mL                       | 0.005<br>( $\times 10^{15}/L^2$ )                       | 0.0012<br>( $\times 10^{12}/L$ ) | Nominal Value of Volumetric Flask<br>1000 mL                           |
| $U_{CC}$ | Coincidence-Correction                 | 751<br>count                    | B    | Normal Distribution                | 2          | 376<br>count                     | 0.000048<br>( $\times 10^{15}/(L \cdot \text{count})$ ) | 0.018<br>( $\times 10^{12}/L$ )  | Coincidence-Correctioned Count<br>105976 count                         |
| $U_{C3}$ | Combined Standard Uncertainty          |                                 |      | Normal Distribution                |            |                                  |   | 0.027<br>( $\times 10^{12}/L$ )  |  |
| $U_3$    | Expanded Uncertainty                   |                                 |      | Normal Distribution<br>( $k = 2$ ) |            |                                  |   | 0.054<br>( $\times 10^{12}/L$ )  |  |

RBC Count assigned by Reference Counter = 5.072 ( $\times 10^{12}/L$ )  $\pm$  0.054 ( $\times 10^{12}/L$ ) , ( $k = 2$ )

## Appendix D. Model equation for measurement and equation of evaluating uncertainty

Equations (1) and (2) in order to calculate RBC concentration are as follows:

$$X = \frac{C_c}{V_M} \times \frac{V_D + V_F}{V_D} \quad (1)$$

where

$$C_c = a + bC_m \quad (2)$$

$X$ : Erythrocyte concentration (/L)

$C_c$ : Corrected count in the diluted sample (measurement unit one)

$V_M$ : Volume aspirated by the manometer (L)

$V_D$ : Volume dispensed by the microdispenser (L)

$V_F$ : Volume of the volumetric flask (L)

$a$ : Intercept of the linear regression equation obtained in advance for correcting for coincidence-loss (measurement unit one)

$b$ : Slope of the linear regression equation obtained in advance for correcting for coincidence-loss (measurement unit one)

$C_m$ : Measured count in the diluted sample (measurement unit one)

By applying the law of propagation of uncertainty, the model equations (1) yield equation (3) shown below.

$$u_c^2(X) = \left( \frac{1}{V_M} \times \frac{V_D + V_F}{V_D} \right)^2 u^2(C_c) + \left( -\frac{C_c}{V_M^2} \times \frac{V_D + V_F}{V_D} \right)^2 u^2(V_M) \\ + \left( \frac{C_c}{V_M} \times \frac{1}{V_D} \right)^2 u^2(V_F) + \left( -\frac{C_c}{V_M} \times \frac{V_F}{V_D^2} \right)^2 u^2(V_D) \quad (3)$$

where

$u_c(X)$ : Combined standard uncertainty of RBC concentration by the reference measurement procedure (/L)

$u(V_M)$ : Standard uncertainty of the volume aspirated into the manometer (L)

$u(V_D)$ : Standard uncertainty of the volume dispensed by the microdispenser (L)

$u(V_F)$ : Standard uncertainty of the volume of the volumetric flask (L)

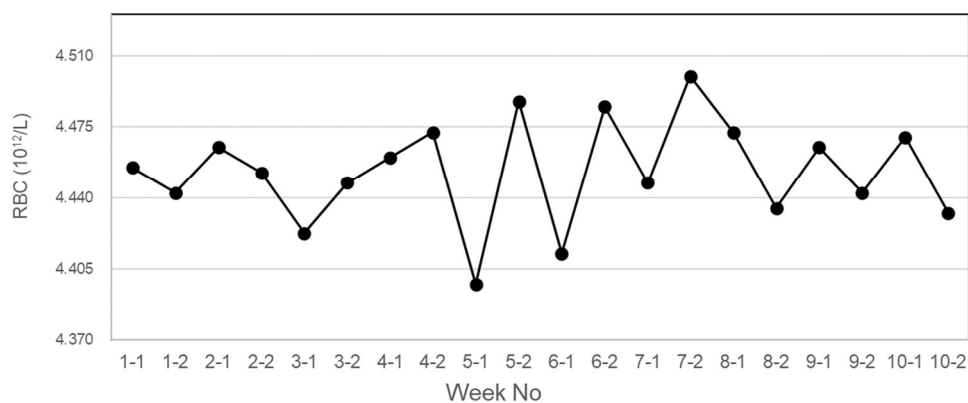
$u(C_c)$ : Standard uncertainty of the corrected count in the diluted sample (measurement unit one)



## Appendix E. Example of internal quality control

| Week No. | Date | RBC  | HCT  | MCV  | Meas. Time |
|----------|------|------|------|------|------------|
| 1-1      | 1    | 4.46 | 39.6 | 88.8 | 26.807     |
|          |      | 4.46 | 39.5 | 88.7 | 26.840     |
|          |      | 4.46 | 39.5 | 88.7 | 26.892     |
|          |      | 4.44 | 39.2 | 88.3 | 26.932     |
| 1-2      | 2    | 4.46 | 39.5 | 88.5 | 26.997     |
|          |      | 4.44 | 39.5 | 88.9 | 27.030     |
|          |      | 4.43 | 40.1 | 90.4 | 27.069     |
|          |      | 4.44 | 40.2 | 90.4 | 27.108     |
| 2-1      | 3    | 4.48 | 40.5 | 90.5 | 26.912     |
|          |      | 4.47 | 40.4 | 90.4 | 26.945     |
|          |      | 4.46 | 40.4 | 90.4 | 26.984     |
|          |      | 4.45 | 40.2 | 90.4 | 27.004     |
| 2-2      | 4    | 4.44 | 39.3 | 88.4 | 26.951     |
|          |      | 4.45 | 39.3 | 88.4 | 26.977     |
|          |      | 4.47 | 39.5 | 88.4 | 27.043     |
|          |      | 4.45 | 39.4 | 88.5 | 27.089     |
| 3-1      | 5    | 4.42 | 39.5 | 89.4 | 26.977     |
|          |      | 4.44 | 39.7 | 89.4 | 26.997     |
|          |      | 4.41 | 39.4 | 89.4 | 27.043     |
|          |      | 4.42 | 40.5 | 91.5 | 27.089     |
| 3-2      | 6    | 4.45 | 39.7 | 89.3 | 26.951     |
|          |      | 4.44 | 39.6 | 89.3 | 26.997     |
|          |      | 4.45 | 39.7 | 89.3 | 27.023     |
|          |      | 4.45 | 40.6 | 91.3 | 27.213     |
| 4-1      | 7    | 4.47 | 40.2 | 89.9 | 26.827     |
|          |      | 4.48 | 40.2 | 89.8 | 26.932     |
|          |      | 4.47 | 40.3 | 90.1 | 26.958     |
|          |      | 4.42 | 40.1 | 90.7 | 26.951     |
| 4-2      | 8    | 4.49 | 40.5 | 90.2 | 26.977     |
|          |      | 4.46 | 40.3 | 90.3 | 26.990     |
|          |      | 4.49 | 40.5 | 90.3 | 27.056     |
|          |      | 4.45 | 40.4 | 90.8 | 27.095     |
| 5-1      | 9    | 4.39 | 39.1 | 89.2 | 27.010     |
|          |      | 4.39 | 39.3 | 89.4 | 27.050     |
|          |      | 4.40 | 39.5 | 89.7 | 27.089     |
|          |      | 4.41 | 39.5 | 89.5 | 27.135     |
| 5-2      | 10   | 4.49 | 40.1 | 89.4 | 27.135     |
|          |      | 4.50 | 40.2 | 89.5 | 27.233     |
|          |      | 4.48 | 40.2 | 89.8 | 27.292     |
|          |      | 4.48 | 40.4 | 90.2 | 27.154     |

Internal quality control: RBC



## Appendix F. Example of interlaboratory comparison

In this example, the results obtained by 6 reference laboratories which are using the reference counters are shown.

| Week No. | Date | Lab. A | Lab. B | Lab. C | Lab. D | Lab. E | Lab. F |
|----------|------|--------|--------|--------|--------|--------|--------|
| 1-1      | 1    | 4.455  | 4.510  | 4.424  | 4.443  | 4.430  |        |
| 1-2      | 2    | 4.443  |        | 4.428  | 4.453  | 4.435  |        |
| 2-1      | 3    | 4.465  | 4.445  | 4.416  | 4.410  | 4.408  | 4.403  |
| 2-2      | 4    | 4.453  | 4.420  | 4.404  | 4.423  | 4.385  |        |
| 3-1      | 5    | 4.423  | 4.358  | 4.380  | 4.420  | 4.403  | 4.393  |
| 3-2      | 6    | 4.448  | 4.378  | 4.392  | 4.433  | 4.398  | 4.380  |
| 4-1      | 7    | 4.460  | 4.453  | 4.394  | 4.445  | 4.430  |        |
| 4-2      | 8    | 4.473  | 4.450  | 4.429  | 4.425  | 4.420  |        |
| 5-1      | 9    | 4.398  | 4.403  | 4.389  | 4.418  | 4.410  | 4.450  |
| 5-2      | 10   | 4.488  | 4.460  |        | 4.410  | 4.458  | 4.450  |
| 6-1      | 11   | 4.413  | 4.385  | 4.508  |        | 4.485  | 4.365  |
| 6-2      | 12   | 4.485  | 4.430  |        |        | 4.480  | 4.410  |
| 7-1      | 13   | 4.448  | 4.428  | 4.504  |        | 4.413  | 4.385  |
| 7-2      | 14   | 4.500  | 4.448  |        |        | 4.430  | 4.393  |
| 8-1      | 15   | 4.473  | 4.415  | 4.571  |        | 4.465  |        |
| 8-2      | 16   | 4.435  | 4.425  |        |        | 4.418  | 4.393  |
| 9-1      | 17   | 4.465  | 4.450  | 4.508  |        | 4.488  | 4.295  |
| 9-2      | 18   | 4.443  | 4.423  |        |        | 4.340  | 4.413  |
| 10-1     | 19   | 4.470  | 4.455  | 4.417  |        |        |        |
| 10-2     | 20   | 4.433  | 4.560  | 4.395  |        |        |        |
| 11-1     | 21   | 4.455  | 4.540  | 4.369  |        | 4.443  | 4.465  |
| 11-2     | 22   | 4.480  | 4.490  | 4.414  |        | 4.455  | 4.425  |
| 12-1     | 23   | 4.455  |        | 4.392  |        | 4.455  | 4.398  |
| 12-2     | 24   | 4.475  |        | 4.402  |        |        | 4.448  |

### Interlaboratory comparison: RBC

