

Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard

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COMMENT

This document addresses calibration and quality control strategies for multichannel hematology analyzers; assignment of values to calibrator materials; calibration using stabilized blood controls; internal quality control; pair difference analysis; and use of the weighted moving average (\bar{x}_w) method.



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Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard

Abstract

NCCLS document H38– *Calibration and Quality Control of Automated Hematology Analyzers*, describes calibration and quality control strategies that will assist in meeting performance goals of multichannel hematology analyzers. The standard is intended for use by clinical laboratory personnel, by reference laboratories, and by manufacturers of hematology analyzers and of calibration and control materials. The document describes errors due to preanalytic variables as well as the establishment of analyzer baseline precision and accuracy. Attention is paid to assigning values to calibrator materials, to analyzer calibration using stabilized blood controls, to using whole blood and pair difference analysis, and to using the weighted moving average (\bar{x}_w) method. The need for participation in external quality assessment (proficiency testing) programs is stressed.

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Foreword

The needs of clinical chemistry established the original patterns of internal and external quality control methodology and the design of control materials and calibrators. Although automated hematology analyzers share these principles, they also have unique characteristics that require a specialized approach to quality control.

Present analyzers appear to have an unrealized potential for automated internal performance checks. Such tests, while not replacing the functional tests provided by whole blood or stabilized blood methods, could adjunctively enhance the control of precision and accuracy.

Advances in international harmonization of the H38 standard are attributed to the expert groups involved in its development, in particular the International Council for Standardization in Hematology (ICSH) cited in the section on **References**. The Expert Panel on Cytometry of ICSH has contributed to H38-P, by prior publications and by fruitful discussions resulting from overlapping membership with NCCLS subcommittees.

Key Words

Accuracy, automated analyzer, bias, blood count, calibration, precision, quality control, reportable range

Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard

1 Introduction

NCCLS document H26– *Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers; Approved Standard* describes performance goals for automated hematology analyzers that would give reliable diagnostic information. This document, a companion volume, describes quality control methodology that will help ensure meeting those performance goals on a daily basis. Among the subcommittee's objectives were improvements in understanding the larger purposes of quality control, and simplification of procedures¹ and statistical interpretations.

Calibration, internal quality control, and external quality assessment of hematology analyzers are largely dependent on stabilized blood products. Therefore, this document offers suggestions to harmonize their specifications with the performance goals of analyzers and with the results given by whole blood quality control methods.

In addition to the use of stabilized blood, data generated by statistical analyses of routine assays can be converted into sources of information for quality control.

2 Scope

This document provides guidance for the performance of calibration and day-to-day quality control of automated multichannel hematology analyzers in the clinical laboratory. This standard also provides supplemental procedures useful for manufacturers and reference laboratories for validating calibration and quality control materials and methods.

3 Standard Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with standard precautions. For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and recommendations for the management of blood-borne exposure, refer to NCCLS document M29— *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

4 Definitions^a

Within the scope of this document, terms used have been defined as follows:^{2,3}

Accuracy, *n* - 1) A measure of agreement between the estimate of a value and a "true" value; quantifiable in terms of departure from accuracy, expressed as systematic error or bias; 2) **Accuracy of an analytic process, *n* -** expressed as the difference between the average result obtainable by a method under specified conditions and the result accepted as true or standard; expressed in the same units as the result, or as a percentage of the standard result (relative accuracy). **NOTE:** a) The lower the difference, the higher the accuracy (the lower the inaccuracy). This difference conventionally includes only process inaccuracy (process bias or systematic error) because the contribution of process

^a Some of these NCCLS definitions are found in NCCLS document H26– *Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers* and NCCLS document NRCL8–*Terminology and Definitions for Use in NCCLS Documents*. For more detailed source information, please refer to these documents.

imprecision (random error) is minimized by the averaging of multiple determinations; 3) **Accuracy of a result, *n*** - Expressed as the difference between a result and the "true" value. **NOTE:** b) This difference includes contributions not only from process inaccuracy but also from process imprecision, especially when one determination per specimen is the rule. It is expressed in the same units as the result; 4) **Accuracy of a control result, *n*** - Identical to definition for "accuracy of a result." **NOTE:** c) However, in this case, because the accepted mean and standard deviation of the population are known, the bias can alternatively be expressed as a Z-score, the signed difference between the result and known mean divided by the known standard deviation. The Z-score is unitless and is a uniform expression for all analytes.

Bias (synonym for "systematic error"), *n* - Systematic, signed deviation of the test result from the accepted reference value; **NOTE:** a) Defined as the difference between the expectation of the test results and an accepted reference value.

Calibration, *n* - 1) The process of testing and adjustment of an instrument, kit, or test system, to provide a known relationship between the measurement response and the value of the substance being measured by the test procedure; 2) The set of operations that establish, under specified conditions the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards; **NOTES:** a) The result of a calibration permits either the assignment of values of measurands to the indications or the determination of corrections with respect to indications; b) A calibration may also determine other metro logical properties, such as the effect of influence quantities; b) A slightly different definition is the set of operations that establish, under traceable conditions, the relationship between values indicated by a measuring instrument or measuring system for an established reference material and the corresponding value of a candidate reference material.

Calibrator, *n* - A (reference) material (e.g., solution, suspension) or device of known quantitative/qualitative characteristics (e.g., concentration, activity, intensity, reactivity) used to calibrate, graduate, or adjust a measurement procedure or to compare the response obtained with the response of a test specimen/sample. **NOTE:** The quantities of the analytes of interest in the calibrator are known within limits ascertained during its preparation and may be used to establish the relationship of an analytical method's response to the characteristic measured for all methods or restricted to some. The calibrator must be traceable to a national or international reference preparation or reference material when these are available. Calibrators with different quantities of analytes may be used to establish a quantity/response curve over a range of interest.

(Quality) control material, *n* - A device, solution, or lyophilized preparation intended for use in the quality control process; **NOTE:** It should be similar to, and is analyzed along with, patient specimens. If different, it should have a recognized, defined response to analytic measurements. Control materials may or may not have known analyte concentrations (i.e., assigned values) within specified limits (e.g., target value \pm standard deviation). Control materials are not used for calibration purposes.

Derived red cell indices, *n* - Quantities (see MCH, MCHC, MCV) that may be calculated from the measurement of hemoglobin (Hb) concentration, packed (red) cell volume (PCV), and erythrocyte (RBC) concentration.

- *MCH* = mean cell (corpuscular) hemoglobin: the average amount of hemoglobin within the red blood cell in a given blood sample.

$$MCH(pg) = \frac{Hb(g/L)}{RBC(x10^{12}/L)}$$

- **MCHC** = *mean cell (corpuscular) hemoglobin concentration*: the average hemoglobin concentration within the red blood cells of a given blood sample.

$$MCHC(g/L) = \frac{Hb(g/L)}{PCV(L/L)}$$

- **MCV** = *mean cell volume*: the average volume of the red blood cell in a given blood sample.

$$MCV(fL) = \frac{PCV(L/L)}{RBC(10^{12}/L)}$$

Linearity, *n* - The ability (within a given range) to provide results that are directly proportional to the concentration {amount} of the analyte in the test sample; **NOTE**: Linearity typically refers to overall system response (i.e., the final analytical answer rather than the raw instrument output (Cf. EP6, EP10). See also **Reportable range**.

Packed (red) cell volume (PCV), *n* - The measure of the ratio of the volume occupied by the red blood cells to the volume of whole blood, expressed as a fraction (L/L). **NOTE**: The term "hematocrit" has been, and is often used for this quantity.

Precision, *n* - The closeness of agreement between independent test results obtained under prescribed conditions; **NOTE**: Precision is not typically represented as a numerical value but is expressed quantitatively in terms of imprecision—the SD or the CV of the results in a set of replicate measurements (Cf. H26).

Quality control (internal), *n* - The set of procedures undertaken in a laboratory for the continuous assessment of work carried out in the laboratory and evaluation of tests to decide whether these are reliable enough for their results to be released to the requesting physician. **NOTE**: The procedures should include tests on control material, results of which may be plotted on a control chart showing upper and/or lower control limits, and may include statistical analysis of patient data. The main object is to ensure day-to-day consistency of measurements or observations, if possible in agreement with an agreed-on indicator of truth, such as a control material with assigned values.

Quantity, *n* - 1) Attribute of a phenomenon, body, or substance that may be distinguished qualitatively and determined quantitatively; **NOTES**: a) The term "quantity" may refer to a quantity in a general sense (length, time, mass, etc.) or to a particular quantity (volume of a given beaker, amount of substance concentration of glucose in a given sample of blood plasma)

Reference material/Reference preparation, RM, *n* - 1) A material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials; **2) Certified reference material, CRM, *n*** - A reference material that has one or more values certified by a technically valid procedure and is accompanied by, or is traceable to, a certificate or other document that is issued by a certifying body; **NOTES**: a) defines a **CRM** as a "reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence"; b) The term "Standard Reference Material (SRM)" is the name of a certified reference material (CRM), which is the trademark name of a certified reference material that has been certified and is distributed by the National Institute of Standards and Technology (NIST), a U.S. Government agency formerly known as the National Bureau of Standards (NBS).

Reference material, certified (CRM), n - A reference material, accompanied by a certificate, one or more of whose property values are certified by an authority from the results of a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Reference population, n - A group of n persons in a described state of health or disease.

Reportable range, n - The value range of assay results within which accuracy is ensured.

Run, n - An interval within which the accuracy and precision of a testing system is expected to be stable, but cannot be greater than 24 hours or less than the frequency recommended by the manufacturer; **NOTES:** a) defines "run" as follows: In a series of observations of a qualitative characteristic, the occurrence of an uninterrupted series of the same attribute is called a "run"; b) Between analytical runs, events may occur that cause the measurement process to be susceptible to variations that are important (Cf. C24).

Sensitivity:

- **Sensitivity, analytical, n** - In this document, 'analytical sensitivity' refers to the smallest quantity of analyte that can be reproducibly distinguished from background noise in a given assay system. It is usually defined at the 0.95 confidence level (2 SD). **NOTE:** ISO's *International Vocabulary of Basic and General Terms in Metrology* defines 'sensitivity' as the change in response of a measuring [system or] instrument divided by the corresponding change in the stimulus. A significant scientific dispute exists regarding this term, its underlying concept and its definition, with the opposing view defining 'sensitivity' in a manner similar to VIM 93's definition for 'limit of detection.'
- **Sensitivity, clinical, n** - The proportion of patients with a well-defined clinical disorder whose test values exceed a defined decision limit (i.e., a positive result and identification of the patients who have the disorder). **NOTE:** The clinical disorder must be defined by criteria independent of the test under consideration.

Set point, n - The level (i.e., analyte concentration) at which an analyzer is calibrated.

Specificity:

- **Specificity, analytical, n** - For quantitative tests, the ability of an analytical method to determine only the component it purports to measure or the extent to which the assay responds only to all subsets of a specified analyte and not to other substances present in the sample.
- **Specificity, clinical, n** - The proportion of patients who do not have a well-defined clinical disorder and whose test values do not exceed a defined decision limit (i.e., a negative result and identification of patients who do not have the disease).

Standard (measurement), n - An authoritative "document" setting forth criteria for performance and characteristics.

- **Standard, primary, n** - A standard that is designated or widely acknowledged as having the highest metro logical qualities and whose value is accepted without reference to other standards of the same quantity.
- **Standard, secondary, n** - A standard whose value is assigned by comparison with a primary standard of the same quantity.

- *Standard, working, n* - A standard that is used routinely to calibrate or check material measures, measuring instruments, or reference materials.

5 General Requirements

5.1 Quality Control Manual

All quality control procedures should be described in detail in a quality manual. This manual may be a part of the procedure manual that describes the methodologies to be used for all assays, with QC methods being separately and clearly indicated. See the most current version of NCCLS document GP2–*Clinical Laboratory Technical Procedure Manuals* for more details. The quality manual should also include a general statement of the laboratory quality policy and an acknowledgment that the policy is understood by all laboratory workers.

There should be a table of organization showing the levels of responsibility for performing various types of QC assays, interpreting the results of these assays, and making decisions regarding them. The persons with authority to design and modify all laboratory procedures and the persons with authority to approve such designs and changes should be listed.

5.2 Record Keeping

Preliminary qualification: Suitable comparisons with previous performance should be provided before analyzing control materials. Calibration protocols should follow established and documented laboratory procedures. Electronic verification and background measurement should be performed and recorded.

CBC/differential files should include control set-up, control run, and control review/report files. Parameters include, as appropriate, RBC, Hb, Hct, MCV, MCH, MCHC, RDW, platelet, and MPV. Any additional RBC parameters unique to any analyzer should meet these same requirements. Differential files should include: WBC (total count), neutrophils (NE[%], NE[#]), lymphocytes (LY[%], LY[#], monocytes (MO[%], MO[#]), eosinophils (EO[%], EO[#]), and basophils (BA[%], BA[#]). Instruments with other special analytic parameters should have files for control set-up, control run, and control review/report.

5.3 Retention of Records

There are three major files of information that are generated in the laboratory. Acquisition and storage of this data base is usually made by main-frame hospital or clinic computer systems.

Laboratory order entry: The laboratory order entry data is maintained for a user-defined number of days and is then purged.

Workload data: Workload data is kept in active files for six months and is then archived. These archives are updated every month and stored in a safe place with timely access to permit review as the need arises. Magnetic or optical storage systems must be validated.

Laboratory data files: The duration of QC record storage depends on local hospital or clinic policies or accreditation requirements, or should be a minimum of two years or more, whichever is greater. Quality assurance records include documented routine maintenance and service, calibration, and control data using commercial products. Proficiency testing results, noncommercial controls, or calibrators, if used, must have complete documentation of standardization and performance characteristics.

5.4 Training and Qualifications

Laboratory workers who are assigned to carry out QC procedures must have been trained in their performance. The laboratory records must indicate this. See the most current version of NCCLS document GP21–*Training Verification for Laboratory Personnel*, for further information. If possible, personnel who are given responsibility for interpreting QC results should not be the same personnel doing the routine work.

5.5 Environment

As far as possible, QC procedures should be carried out in an environment that reflects routine working conditions. Particular attention should be given to stability of ambient temperature. A record of temperature in the work area and in the storage areas, including refrigeration, should be maintained. Any unusual change in environmental conditions should be noted and corrective action taken.

5.6 Analyzer Identification

The laboratory must maintain a record of each analyzer that will include, as a minimum, the following information:

- Laboratory name and address.
- Manufacturer name and address.
- Model type and serial number.
- Date of installation.
- Identification of person(s) involved in the installation of the analyzer.
- Narrative of specific installation problems including solutions.
- Dated and signed documentation of final installation status.
- Dated and signed log of system updates.
- Dated and signed log of repairs, including description of parts replaced.
- Dated and signed log of analyzer problems and description of steps required for resolution.
- Name(s) and qualifications of key operator(s).

5.7 Preanalytic Variables

Uncontrolled preanalytic variables can exert a misleading effect on estimates of imprecision and bias. Operators should be made aware of these sources of error and be prepared to guard against them. A checklist of possible error sources will help ensure that they will not be overlooked. The principal preanalytic variables are discussed below.

5.7.1 Analyte Instability

Analyte instability usually shows as a progressive temperature and time-dependent, unidirectional change in results, not affecting all analytes equally. It has the effect of giving a nonrandom character to data that is to be used for the iterative (repeated assay) method for measuring imprecision. It is least likely to be seen in stabilized blood preparations and most likely to be seen in recently collected blood specimens. Over-vigorous mixing may also play a role.

Viral disease may cause reduced lymphocyte count due to cell fragility. Cold agglutination (Section 5.7.3) may affect platelets as well as red cells.

Detection of analyte instability depends on the demonstration of a difference between the mean value of the first half of an iterative assay sequence and the mean value of the second half. To be significant the difference between means should exceed standard error of the first mean at 95% confidence. More sophisticated methods for the detection of non-randomness can be found in statistics texts, but comparison of means has been found to be suitable for the purpose.

Since this effect is time-dependent, its magnitude will depend on the duration of the test, the number of iterations and interruption and prolongation of the test by routine laboratory work.

5.7.2 Analyte Nonuniformity

This is usually caused by inadequate mixing. Fresh blood is less susceptible to this error than stabilized blood but is less able to withstand the mechanical stresses of mixing. Mixing a specimen by rapid shaking or vortexing or any method that causes frothing should be avoided.

There are two components in nonuniformity. One is the nonuniform dispersion of the total cell mass throughout the plasma. The other is the nonuniform distribution of the different cell types with respect to each other. In order to achieve uniformity of a whole blood suspension, make an initial ten complete inversions of the specimen container. A completely filled container gives poor mixing; an air bubble must be present. If the specimen has been stored for 2 to 5 minutes, three additional inversions should precede the assay.

Manufacturers of stabilized blood products should provide exact directions for the manner in which mixing and remixing should be carried out.

5.7.3 Autoagglutination

Autoagglutination is a cause of unstable suspensions. If not visible to the eye, autoagglutination or rouleaux should be suspected if 1) reduced RBC together with elevated MCHC are encountered; 2) the MCV may be slightly to markedly increased; 3) the rule of thumb " $3 \times \text{Hb (g/dL)} = \text{Hct (\%)} \pm 3$ " is not met. Its presence can usually be confirmed by microscopy, but be aware that a warm slide may cause autoagglutinates to dissociate. Specimens with autoagglutination should not be used in quality control procedures.

Refrigerated specimens should be brought to room temperature before analysis.

5.7.4 Anticoagulant Effect

Fresh blood is more susceptible to anticoagulant effects than preserved blood. Insufficient blood collected into an evacuated container may cause shrinkage of all cell types, but red cells usually become restored to the size dictated by their osmotic equilibrium with the cell-counting diluent.^{1,4} Leukocytes, erythrocytes, and platelets (e.g., EDTA-induced aggregation) may be irreversibly changed and cause errors in the differential count and platelet count and MCV. The analyzer product reference manual should be consulted for the manufacturer's warnings and limitations regarding anticoagulant types.

6 Establish Baseline Parameters

The analytical baseline data provide reference levels for judging the performance of the analyzer subsequent to their measurement. Baselines should be established or verified when a new analyzer is installed and when a modification or repair is made that could significantly affect the analyzer performance. The product reference manual should provide instruction for frequency of recalibration under normal operating conditions. Calibration or recalibration must be preceded by verification of the major analytical variables, which are general conditions of imprecision and accuracy and the analyzer. Reportable range should be verified for a new installation and after major repair. To avoid the possibility of analyzer defect causing errors in the baseline procedures, preventive maintenance should be performed shortly before calibration.

6.1 Imprecision Baseline—Specimen Selection

Baseline imprecision is described by the standard deviation of replicate assays of the same specimen. (See the most current edition of NCCLS document EP5— *Evaluation of Precision Performance of Clinical*

Chemistry Devices.) The specimen may be fresh blood or stabilized blood. Measurement of imprecision should be made at high, normal, and low analyte levels. With fresh blood, low values can be made by diluting the specimen with its own plasma. High values of concentration-dependent analytes can be made by sedimentation with the specimen tube at a 45° angle for two hours, removal of one half the plasma, and remixing. Analytes that are not concentration-dependent, e.g., MCV, MCH, MCHC, RDW, MPV, and PDW may require a search for unique specimens.

Stabilized blood preparations should not be modified in the laboratory.

6.2 Measure Baseline Imprecision

Make n consecutive assays of the same well-mixed fresh blood or stabilized blood. The preferred number of iterations is $n=31$. Fewer repeated assays (for example, $n = 10$) may be used, but if this is done, convert standard deviation (SD) to confidence limits (CL) as described in **Section 6.2.1, Evaluate Baseline Imprecision**. Record the results of each analyte assay in a table similar to Table 1. Note the need to record the elapsed time of the measurement.

The standard deviation of each analyte is calculated from the formula:

$$SD = \sqrt{\frac{\sum [x_i - X_a]^2}{n-1}}$$

where:

x_i is the result of an individual assay,
 X_a is the mean of n assay results,
 n is the number of repeat assays, and
 SD is the standard deviation of the data set.

Coefficient of variation (CV) may be calculated from the following formula using the same terms:

$$CV\% = \frac{SD}{X_a} \times 100$$

It is recognized that most, if not all, data collection, analysis, and imprecision measurement may be carried out by a computer that supports the operation of the analyzer. Table 1, in addition to providing a model for manual analysis of imprecision, shows a pattern that should be followed by a computer. In cases where the number of replicated assays is beyond the operator's control, it is recommended that SD calculated from n less than 31 be converted to confidence limits (see Section 6.2.1).

Table 1. Suggested Layout of Results from Measurement of Imprecision by Replicate Assays

| | | | | | |
|---|----------|-----|----|-----|-----|
| Date ../../.. Time started : am/pm Time completed : am/pm | | | | | |
| Technologist..... | | | | | |
| Material source. Fresh blood ID..... Stabilized blood Lot#.....Exp date..... | | | | | |
| Assay # | Analytes | | | | |
| | WBC | RBC | Hb | MCV | Plt |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5,6,7, etc. | | | | | |
| 30 | | | | | |
| 31 | | | | | |
| Mean | | | | | |
| SD | | | | | |
| CV | | | | | |

6.2.1 Evaluate Baseline Imprecision

Imprecision may be stated as SD or $CL_{95\%}$. (95% confidence limit). If comparison is to be made between standard deviations from different sources, e.g., between the laboratory estimate and a manufacturer's labeled value, be sure that each measurement has been made with the same number of iterations (n). If different values of n have been used, convert SD to $CL_{95\%}$ as follows:

Multiply each SD by the value of t for $(n-1)$ given in the Appendix. For example, the laboratory used $n = 31$, but the manufacturer's labeled SD is based on $n = 16$. Therefore, multiply the laboratory SD by 2.0423 and the manufacturer's SD by 2.1315. This will convert both SD's to $CL_{95\%}$, making it possible to compare them.

The laboratory CL should not be greater than the manufacturer's CL. If this condition meets this requirement, calibration may proceed. If not, there should be an investigation to determine the cause of the increased imprecision, and corrective action should be taken before proceeding with calibration.

6.3 Accuracy Baseline

The accuracy baseline is defined as the starting point against which future bias control information will be compared. Verification of accuracy must be made immediately (less than 15 minutes) after calibration or as directed by the manufacturer. If delayed, the data from the baseline verification process may be contaminated by drift or other analyzer error.

The term "calibration" means adjusting the analyzer to achieve zero bias, or verifying through a similar process that zero bias already exists. The term "zero bias" means that any difference between the calibrator-assigned values and the results of calibrator assay is due only to analytical imprecision.

It is assumed that all automated hematology analyzers have a linear response over the reportable range, that the limits of linearity are specified, and that zero analyte concentration produces a zero analyzer response. These characteristics make it possible to calibrate analyzers by a single-point method.

6.3.1 Stabilized Whole Blood Calibrator Characteristics

Calibrators made from stabilized human and/or animal blood enjoy wide and generally successful use. Stabilized blood controls with assigned values are sometimes unwisely used as less costly substitutes for calibrators. Although calibrators and controls from the same manufacturer may have a superficial resemblance, there may be sufficient differences in manufacturing processes, labeling, assignment of values, performance control, and postmarket surveillance to make calibrators and controls noninterchangeable.

Calibrators should have the following characteristics:

*Assigned values:*⁵ These should be conferred by transfer of whole blood reference values as shown in Section 8. They should not be conferred by transfer of values from prior lots of a calibrator nor by transfer of values from the calibrator of another manufacturer. The levels of assigned values should be chosen to provide the best compromise between the benefits of high concentration (good resolution and lower relative Poisson error) and the disadvantages (high viscosity and possible nonlinearity).

Stability: The assigned values should not change during the labeled shelf life. If there may be changes after opening the vial, the product labeling should so state.

Labeling: The labeling of a calibrator should identify the types and models of automated analyzers for which it is suitable and should caution against its use with nonlisted analyzers. The table of assigned values should include CL_{95%} of each value. The labeled method of use should not be in conflict with the calibration procedures recommended by the analyzer-manufacturer. Any analytical disparity between reference values and analyzer values should be described in the package insert and its clinical implications discussed.

6.3.2 Reference-Assayed Whole Blood Calibration

The subcommittee appreciates that direct whole blood calibration of automated analyzers is rarely used today in medical institutions. However, it is the cornerstone of the recommended method for assigning values to stabilized blood calibrators. Its use for this purpose is discussed in Section 8.

6.3.3 Stabilized Blood Calibration Method

Frequency of calibration: Follow the recommendation in the analyzer product reference manual. Usually calibration is recommended after a major repair or after a significant change of base line accuracy (drift). In the latter case, the cause of excessive drift should be corrected before proceeding with calibration.

Iteration of calibrator assays: The number of assays will influence the precision of the calibration factor. Since the SD of the analyzer has already been determined, it is useful to estimate the error range of the calibration factor by use of standard error of the mean (SEM) formula below. Greater assay iteration will result in a lower SEM.

$$SEM = \frac{SD}{\sqrt{n}}$$

SD is the standard deviation of the analytical method, and n is the number of replicated assays.

More replication will yield a more precise calibration set point and more accurate subsequent patient assays but, because of the square root function, the rewards of increasing replication will be on a scale of diminishing returns. Furthermore, excessive mixing associated with increased replication may damage cells and introduce other errors. It is suggested that ten consecutive assays of the calibrator will give a satisfactory result. For example, the SD for WBC was found to be $0.2 \times 10^9/L$. The SEM of ten calibrator assays will then be $0.2 \div \sqrt{10}$ or $0.2 \div 3.16 = \pm 0.063 \times 10^9/L$. This error, in combination with the analyzer SD will be propagated (included in) to patient assays. To obtain the 95% assay bias range, use the root sum square variance method given below.

$$Range = 2(\sqrt{SD^2 + SEM^2})$$

6.3.4 Record Calibration Results

It is recognized that most, if not all, data collection, analysis, and calculation of calibration set point may be carried out by a computer that supports the operation of the analyzer. Table 2, in addition to providing a model for manual analysis of set point, shows a pattern that should be followed by these computers. In cases where the number of replicated assays is beyond the operator's control, it is recommended that SD calculated from n less than 31 be converted to confidence limits (see Section 6.2.1).

Table 2. Suggested Layout for Recording Calibration Results

Calibrator Mfr. & Lot # Exp Date/...../..... Date opened/...../.....
 Technologist Analyzer ID
 Date of calibration/...../..... Time started : Time completed :

| Sequence | WBC | RBC | Hb | MCV* | Plt |
|-------------|-----|-----|----|------|-----|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| Mean © | | | | | |
| Cal Val (R) | | | | | |
| C ÷ R | | | | | |
| Analyzer SD | | | | | |
| SEM | | | | | |

*Note that some analyzers require Packed Cell Volume (PCV) to be used instead of MCV.

Divide the mean of the calibrator results © by the calibrator assigned value (R) for each analyte to derive the calibration factor. The calibration factor should be calculated and recorded whether or not the analyzer has automatic adjustment. If $C \div R > 1.0$ the current calibration factor must be proportionately adjusted upwards. If $C \div R < 1.0$ the current calibration factor must be proportionately adjusted downwards. Some analyzers can automatically adjust to make the analyzer set point equal to the calibrator assigned value. This method employs additional significant digits and removes the human error of calibration adjustment.

6.3.5 Set Point Confidence Limits

The laboratory will find it useful to attach a 95% confidence limit statement to the calibration factor. If the laboratory finds that zero bias (calibration factor = 1.00) is within the confidence limit, the current set point is statistically the best that can be achieved. Prudent use of confidence limits will reduce the number of recalibrations. The calibration confidence limit ($CL_{95\%}$) is obtained by combining the confidence limit of the calibrator assigned values with the confidence limit of the analyzer for each analyte by the root sum squares method. An example of the calculation used to derive the confidence limit of the calibration set point is given in the Appendix.

Analyzers that require manual set-point adjustment will carry a higher probability of bias than analyzers that are self-adjusting. If unacceptable bias is discovered in the verification stage in this type of analyzer, there is little remedy but to repeat the calibration process with more care.

6.3.6 Verification of Set Point

It is necessary to confirm (verify) that calibration has been successful. The analyzer product reference manual should provide a recommended procedure. If no alternative method is given in the manual, repeating the calibration procedure is recommended.

6.3.7 Recover Calibrator Values

This form of verification consists of repeating all calibrator assays immediately after bias adjustment has been made. The measure of the agreement between calibrator assays and verification values is the mean difference between those two data sets. This mean difference will rarely be zero, since it will be influenced by analytical imprecision, but if it falls within the combined confidence limits of the calibrator, the calibration process, and calibration verification, a practical unbiased condition will have been achieved. If the mean difference exceeds the upper or lower boundaries of these limits, all future assays must be assumed to be biased. Whether or not bias thus disclosed should lead to recalibration depends on the laboratory's action limit policies contained in its procedure manual.

This method provides proof only that calibration has been satisfactorily performed *with respect to the calibrator*, within the confidence limit of the calibrator assigned value (CL_{cal}) combined with the confidence limit of the analyzer ($CL_{analyzer}$), and the confidence limit of the calibration process ($CL_{calibration}$), according to the following formula:

$$CL_{calibration} = \sqrt{CL_{cal}^2 + 2CL_{analyzer}^2}$$

NOTE: The expression 2CL is derived from the assumption that CL of the analyzer is equal to the CL of the calibration assays.

Recovery of calibrator values does not confirm absolute truth. If the calibrator has degenerated, or if the assigned values are erroneous, in future assays of patient specimens will reflect such information.

6.3.8 Verification of Noncalibratable Methods

Current (1997) designs of automated leukocyte differential counters that measure the internal ratios of the different cell types do not require calibration. However, periodic verification of the accuracy of cell classification should be performed. The reference method and the comparison procedures described in NCCLS document H20–*Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods*, are suitable for this purpose.

Bias adjustment may not be provided in automated reticulocyte counters, since these analyzers express reticulocytes as a proportion of the red cell count. Verification of the red cell/reticulocyte ratio should use the reference method and comparison procedures given in NCCLS document H44–*Methods for Reticulocyte Counting (Flow Cytometry and Supravital Dyes)*. Accurate RBC values are needed to provide absolute reticulocyte counts. The RBC calibration and calibration verification rules given above will apply to the source of this information.

Consult the product reference manual for suggested corrective action if verification is not achieved for any noncalibratable method.

6.4 Reportable Range

Reportable range is the range of assay values over which accuracy is ensured; hence its reportable to the patient's record. It describes only the effect of analyte concentration and does not include possible error due to interferences, such as agglutination or hemolysis. Upper and lower limits of reportable range for a newly installed analyzer can be verified by recovery of the assigned values of abnormal high and abnormal low stabilized blood controls. Assays of sequential dilutions of blood between these limits do not contribute information that is helpful in establishing extension of this reportable range. Reassurance of midrange linearity is given by the recovery of the assigned value of the normal stabilized blood control and by the verification of the calibration set point.

It is recommended that analyzer manufacturers include reportable range limits as a part of analyzer specifications.

6.4.1 Verify Lower Limit of Reportable Range

Supporting data for extending the lower limit of the reportable range, beyond that given by the abnormal low control, can be obtained by diluting a normal blood specimen with its own, cell-free plasma. A volumetric pipet, or dropper pipet held vertically, its tip not touching the side of the vessel, may be used to prepare dilution(s) of normal blood to reduce the concentration of all analytes to values that the laboratory defines as its lowest reportable range limit. The dilution factor is given by the following formula:

$$Dil = \frac{Bl}{(Bl + Pl)}$$

where:

Dil = Dilution factor,
Bl = Volume or drops of blood, and
Pl = Volume or drops of plasma.

Resuspend diluted and undiluted specimens and make four assays of each. Multiply the mean of the results of the four whole-blood assays by the dilution factor. Compare this result to the mean of the results of four diluted-specimen assays. Lower-limit extension of the reportable range will have been achieved if the two sets of means agree within their combined confidence limits. For the normal blood specimen, use the limits previously measured for normal stabilized blood control. For the diluted specimen, use the two times the limits previously measured for abnormal low stabilized blood control (Section 6.2). This multiple arbitrarily assigns the value of the low abnormal control CL to the dilution process.

The formula for calculating the CL of the lower reportable range limit is as follows:

$$CL_{limit} = \pm \sqrt{2CL_{low}^2 + CL_{norm}^2}$$

where:

CL_{limit} = confidence limit of lower reportable range limit,
 CL_{low} = confidence limit of abnormal low control, and
 CL_{norm} = confidence limit of normal control.

6.4.2 Verify Upper Reportable Range

There are two methods that may be used to extend the reportable range above the limit established by the high abnormal stabilized blood control:

- (1) Verify that an elevated analyte concentration, observed in a patient's specimen, is reportable by the dilution method given in Section 6.4.1. When further abnormally high analyte cases are encountered, a list of extended ranges may be developed by applying the dilution method as each occasion arises; or
- (2) Concentrate the cells in a normal specimen by sedimentation for two hours in a tube sloped at 45° (see Section 6.1). Remove sufficient plasma to achieve the required analyte concentration. Verify accuracy of the concentrate assays by quantitative dilution described in Section 6.4.1 and exemplified in the Appendix. This method will not provide the selective concentration needed for verifying upper WBC and platelet limits, unless abnormal specimens with elevated values of these analytes are used. Selective concentration of WBC and platelet can be made by differential centrifugation.

7 Purpose and Methods of Internal Quality Control

Once a performance baseline has been established, its control is based on methods that will detect loss of accuracy or loss of precision, or both. Control methods must be sensitive enough to reveal loss of performance that could compromise patient assay values, but they cannot be oversensitive to the point of signaling error when none exists. NCCLS document H26–*Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers*, gives performance goals that were defined in terms of the clinical consequences of error. Those criteria gave guidance for the sensitivity and specificity of performance control systems. As a minimum, a performance control system must provide the following information:

- Analytical imprecision has not deteriorated with time or use.
- Bias has remained within the limits established at the time of calibration.

The term "internal" indicates quality control procedures that take place within the laboratory immediately before, during, or immediately after an analysis run. Please refer to the most current version of NCCLS document C24– *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*, for further information. The procedures must be carried out as an integral part of patient assays. The definition of a "run" should be related to work loading and to the stability (drift) of the analyzer, not simply to elapsed time. Control assays performed only once daily may not provide enough information to ensure accuracy and consistency of results. When the analyzer is restarted after complete shut-down, precision and set point should be verified to ensure that satisfactory analytical capability has not been lost.

Internal quality control is distinguished from external quality control. The latter is administered by a third party, such as an authorized proficiency testing body, based on blind assay of a stabilized control material, or by a manufacturer offering statistical peer comparison of routine stabilized blood control results.

Analyzers of differing designs and operating principles will have differing propensities for failure or for performance deterioration. All available methods of error detection, (e.g., weighted average, pair difference), should be employed as adjuncts to stabilized blood preparations.

7.1 Quality Control Using Stabilized Blood

Stabilized blood controls should be similar to calibrators (see Section 6.3.1), except that assigned values, if provided, may be conferred by calibrated analyzers using stabilized blood calibrators instead of reference-assayed whole blood. However, the purpose of quality control tests, namely the detection and measurement of change, can be achieved with laboratory-assigned values. Manufacturers' assigned values pose a temptation to use controls as calibrators and to "unblind" some types of internal and external performance reporting.

Controls should have two levels of analyte concentration. These levels should reflect performance at normal analyte levels and at, or close to, the low limits of the reportable range.

Stabilized blood controls must be labeled for use with specified analyzers. The laboratory should document that the product it uses is so labeled.

7.1.1 Frequency of Use

Decide how many assays should separate controls. This decision should be made on the basis of work load and experience with the drift characteristics of the analyzer(s). If the results of daily control assays are within laboratory-defined limits, such as ± 2 SD of the assigned or conferred value, it can be assumed that the results of patient assays performed within a control-bracketed work segment are valid and reportable. The fewer the control assays relative to patient specimens, the greater will be the number of assumed invalid results in the event of an out-of-control condition. As a minimum, assays of normal and abnormal levels of control material should be made at the beginning of each run and one assay of normal level control made at the end of the run to ensure that patient results have been satisfactory. Further assurance of performance can be given by additional control assays made during the run.

7.1.2 Test Replication

The mean of replicate assays of control material is more informative than a single measurement. This principle has been discussed in Section 6.3.3. Paired assays are recommended. Not only does the mean of the pair give a more exact value, but the difference between members of a pair also gives an estimate of imprecision.

Overlap is a special case of test replication. When a new lot of control material is received, it should be tested in parallel with the currently used lot for four days. This will establish the ratio of the assigned values of the incoming lot to the assigned values of the currently used material. Differences in assigned values of the two lots should not be taken as an indication to recalibrate.

7.1.3 Quality Control Methodology

The basis of the method is continued conformance of control assay values to the assigned values of the stabilized blood product. These values may be manufacturer-assigned or assigned by the laboratory, using an analyzer that has satisfied the requirements of base line accuracy (see Section 6.3).

Recovery of assigned values within the confidence limits of the analytical process, combined with the confidence limits of the assigned values, will provide assurance that analyzer accuracy and precision are acceptable. Any disagreement that exceeds these expected range limits calls for further investigation to determine whether it is a random event, loss of precision, loss of accuracy, or deterioration of the control material. The laboratory should establish its own expected ranges of recovered control values as described in Section 6.2. These ranges will usually be smaller, hence more informative, than the manufacturer's published ranges. It is recommended that result recovery ranges be expressed as ± 2 SD, or preferably $CL_{95\%}$ of the mean value.

7.1.4 Interpretation of Results

An interpretive method for out-of-limits control result should be able to distinguish among the following: random event, loss of precision, and loss of accuracy.

Random event versus imprecision: With control boundaries defined as ± 2 SD, one control value in twenty will exceed these limits as a random event. Exclude random event by two further assays of the same type of control (normal, high, or low) that gave the out-of-limit result. If the difference between these assay results is less than the established ± 2 SD limits, the out-of-limit control result was probably a random event and requires no further action.

If the difference is greater than ± 2 SD, assume precision loss and confirm this by two further assays. At least two of the total of five results will exceed ± 2 SD limits if precision has deteriorated. In this case, patients' results that preceded the out-of-limit condition should be reviewed in order to determine reportability.

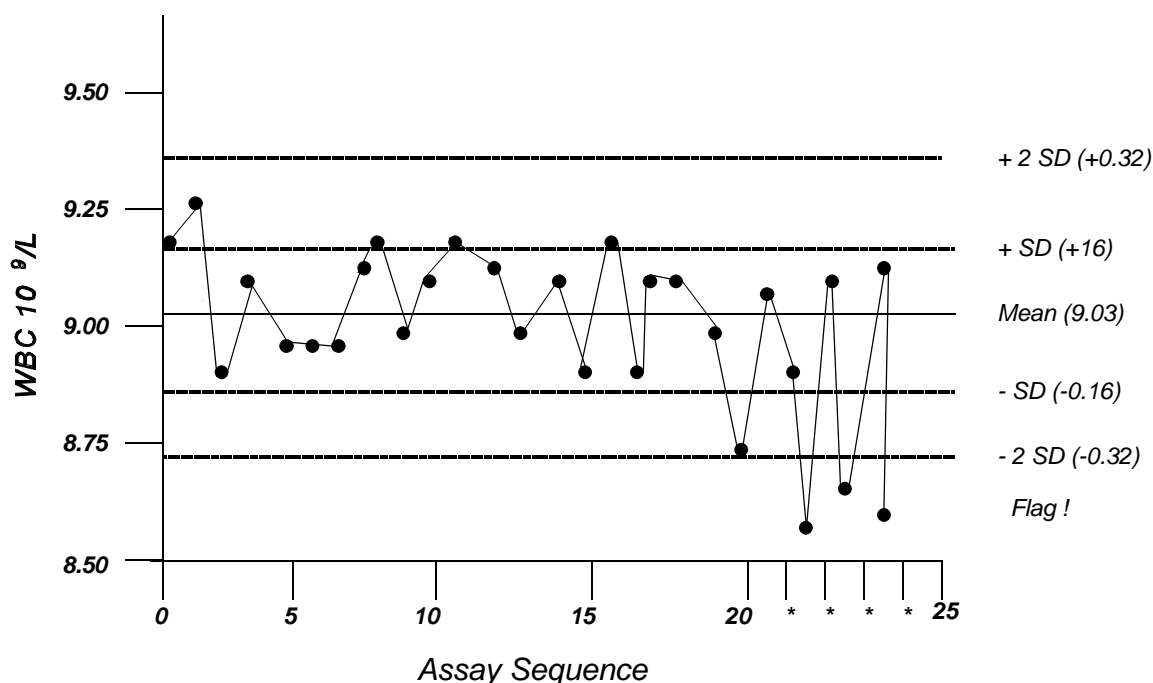
Loss of accuracy: An out-of-limits control result due to loss of accuracy (bias change) can be verified by the repeated assay method given above. If inaccuracy has developed, the mean of the replicate results will differ by at least 1 SD from the mean of previous control results. If the bias change affects only one of the three control levels, suspect a deterioration of the control material. If the bias change affects the red cell indices, inspect the weighted moving average values for supporting evidence of change in RBC, Hb, or MCV.

NOTE: In this section (7.1.4) when the term "SD" is used with the precursor \pm (\pm SD) it describes the dispersion of values around the mean. When used without the precursor (SD), it describes the magnitude of separation of data *points* regardless of the mean of the data *set*.

Example. Figure 1 is a Levey-Jennings plot of a sequence of results for the normal WBC control. The 25th result falls below the lower -2 SD limit, which initiates an investigation into possible causes. Results 26 to 29 (marked by ****) are sequential additional control assays.

Figure 1. Levey-Jennings Chart WBC Control

The investigation shows that the results of assays 26 and 27 are separated by more than 2 SD (0.32)



which suggests that the problem may be a loss of precision. Assays 28 and 29 confirm that precision is compromised. Also, the new mean of the most recent set of five control assays is 8.8 versus the previous mean of 9.03, a significant change.

The conclusion drawn is that there is a loss of both precision and accuracy. It was noted that the normal control material is approaching its expiration date. A check of the abnormal low and high controls showed that they gave recovery of the assigned values within the expected limits. The final conclusion was that the normal control material has deteriorated but that there was no loss of analytical accuracy of patient samples.

7.1.5 Computer Management of Results

Manual compilation and review of control results is time- and labor-intensive. Therefore current (1999) multichannel automated hematology analyzers should store the results of stabilized blood assays in computer files, perform quality-related calculations, and flag results that violate laboratory-assigned limits. (See the most current edition of NCCLS document GP19— *Laboratory Instruments and Data Management Systems: Design of Software User Interfaces and End-User Software Systems Validation, Operation, and Monitoring*.) In order to meet user needs, automated quality control systems should have the following capabilities:

- **System capacity:** The system capacity should be three times the number of control assays performed during the control material time cycle. If three levels of controls are used, three additional files should be available to store the next cycle of incoming control data and also permit storage of overlap assays. There should be running visual indication of the remaining file capacity.
- **Identify control material:** The system should be able to uniquely identify control material by name, lot number, nominal analyte level (low, normal, and high), expiration date, and acceptable

recovery ranges. Manufacturers of stabilized blood controls usually provide suggested recovery ranges, but good practice dictates that the laboratory establish its own ranges.

- *Other control materials:* Provide data storage for QC materials from other commercial sources or locally developed QC materials to permit contemporaneous analysis with ongoing commercial QC materials. Store results of external QC and proficiency test programs.
- *Whole blood control systems:* Store RBC indices values from selected patient assays. Perform weighted moving average calculations and display results graphically (see Section 7.2).
- *Store results:* Control assay results should be captured and stored. This information should also include: date and time of assay, technologist identification, and flags indicating questionable performance, determined and stored for comparison purposes over a specified number of days appropriate for the stated stability of each QC material.
- *Overlap:* There should be provision for standardizing the number of overlapping assays that bridge the preceding and succeeding lots of control material and for verifying the recovery of assigned values. Four days of overlapping results are recommended.
- *Review control results:* Immediately after a control has been assayed, the system should review the results and notify the operator of any values that exceed the stipulated recovery range. It is desirable for the system to be able to record (write to file) the operator's acknowledgment of, and responsive action to, out-of-range values.
- *Review patients' results:* Transmission of results to a host computer should be under laboratory authority. Detection of an out-of-control condition should allow immediate review and decision regarding release of preceding results.
- *Expected range limits:* The control assay value limits that indicate loss of precision or accuracy should be set by the laboratory.
- *Displays:* On request, the system should be able to provide viewing and printing of control recovery data. These data should include a listing of values versus time, a scatter-plot of recovered values versus time, and comparative viewing. That is, adjacent plots for the different control levels.
- *Special analysis:* On request, perform special analysis of control results, e.g., trend analysis, and constancy of codependent measured and calculated values, such as the codependency of MCH and MCHC on Hb, RBC, and MCV.
- *Back-up:* The system should automatically protect quality control files by periodic back-up, for example nightly, by downloading to the laboratory host computer or diskette.
- *Downloading:* If the manufacturer offers an interlaboratory (external) quality evaluation or proficiency program, electronic downloading is a helpful feature of the program. The download should include a step to verify accuracy of the read/write function.
- *Show calculation methods:* Statistical calculations should be described in the product reference manual. See also *whole blood control systems*, above.
- *Long-term storage:* The system should provide long-term storage of control results, either by printed hard copy or by separate magnetic disk that can be stored without deterioration for the institution's stipulated period. Optical disk methods may, at present, require legal approval.
- *Internal diagnosis:* The subcommittee recommends (see Foreword) that manufacturers consider using the computer to monitor the internal physical variables of analyzers.

7.2 Quality Control Using Patient Results

In addition to the use of stabilized blood, data generated in the course of routine assays can be converted into sources of information for quality control. These are shown in Table 3.

Table 3. Uses of Whole Blood for Quality Control

| Method | Purpose |
|---------------------------|-------------------------|
| Pair comparison | Imprecision control |
| Weighted moving average | Short-term bias control |
| Reference interval change | Long-term bias control |
| Manual differential count | Verify specificity |

Pair comparison is a method for monitoring change of precision. It helps to determine whether changes in stabilized blood control values are the result of imprecision or change of bias. It may be used in two ways - *pair difference analysis* and *within run comparison*.

The weighted moving average method^{6, 7} is based on the fact that changes in the repeatability of red cell indices assay results is more likely to be caused by analytical factors than by biological factors. The formula for weighted moving average calculation minimizes the effect of newly entered results on the mean of a batch (usually 20) of patients' values.

The importance of reference intervals in relation to clinical goals has been discussed in NCCLS document H26- *Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers*. Reference interval changes may be the result of analytical bias but may also be caused by changes in population demographics.

7.2.1 Pair Comparison

Pair difference analysis: The SD of the differences between pairs of patient assays gives a measure of comparative analytical imprecision. Ten specimens per run should be duplicated. Using more duplicates increases the robustness of each value of SD, thus making the test more sensitive, but also more costly in reagents. The same number of duplicates must be used for each run in order for the SDs of each set to be comparable.

Establish documented rules for the selection of specimens to be paired. As far as possible, space the duplicate specimens evenly throughout the run. The standard deviation of pair differences may not be numerically equivalent to standard deviation measured by replication, because the precision of analyzer cell counting channels is affected by analyte concentration. Therefore avoid specimens having markedly abnormal values. Falsely elevated differences may be caused by auto-agglutination or inadequate mixing. Be aware of the possibility of interaction (carry over) between the first member of a pair and the specimen that precedes it.

Table 4. Suggested Layout for Recording Pair Difference Analysis

| Specimen ID. | Assay A | Assay B | A minus B |
|---------------|---------|---------------------|-----------|
| 1 | | | |
| 2 | | | |
| 3, 4, 5, etc. | | | |
| 9 | | | |
| 10 | | | |
| | | Mean of differences | |
| | | SD of differences | |

Calculate the standard deviation of the pair differences column using the SD formula given in Section 6.2.

Full implementation of this imprecision monitoring method requires a suitably programmed computer with automated data acquisition capability.

Within-run comparison: Repeat assays of a single specimen, interspersed with routine single assays, will provide an indication of repeatability. Rather than attempting to calculate a dispersion index (SD) for this small number of assays, check that the difference between the results of any pair of these interspersed assays is within established analytical confidence limits.

7.2.2 Weighted Moving Average (\bar{x}_B) Method

This is a method for monitoring bias changes of red cell indices, MCV, MCH, and MCHC. Its effectiveness depends on the biological stability of red cell indices. That is, an apparent change of any of these analytes in an individual is more likely to be due to analytical error than to physiological factors.⁶ The method is practical, because hematology analyzers now provide automated data acquisition and computation.⁷

Patient assay results are automatically entered into a formula that gives a running mean and progressively compensates for variations among incoming results. Data batch sizes of $n = 20$ are suitable for providing stable means for comparison with target values. Although the formula “smooths” the results, it is advisable to minimize the use of clinical specimens having unusually high rates of RBC index dyscrasia. This is because some diagnoses may be associated with minor, systematic but significant abnormality of indices and should thus be avoided. In some geographic locations, marginal nutritional deficiency may cause a properly calibrated analyzer to give local target values that are different from published norms. This finding should not lead to recalibration. The strength of the method lies more in its ability to detect bias change than in the absolute reported values of a local population.

Batch means are calculated and displayed and/or printed for review. RBC parameters (RBC, Hb, Hct, MCV, MCH, MCHC) are stored in the \bar{x}_B files. Statistical comparison of mean values with target values for within-limits performance is determined and displayed for review. Flags will be provided for mean values that are out of \bar{x}_B limits. Provision for editing outlier data points is provided. A reasonable goal for recognition of change is a 3% departure from a previously established and verified target value.

7.2.3 Monitor Reference Intervals

Periodic review and verification of the reference intervals of all analytes is recommended. Methods for the selection of subjects and analysis of results are given in NCCLS document C28– *How to Define and*

Determine Reference Intervals in the Clinical Laboratory. Although loss of accuracy will be reflected by a change of reference intervals, the acquisition of a sufficiently large database to make a short-term bias statement is impractical. If a change in reference intervals is detected, other methods discussed above provide greater sensitivity and real-time information.

7.2.4 Monitor WBC Differential

Manual differentials can be part of overall quality control, as defined by local, state, or federal protocols. Provisions should be made for data entry of manual cell identification data for comparison with analyzer cell identification. Manual and analyzer values should be collected for an appropriate number of days, and the mean differences and standard deviations for each cell type should be determined and compared to laboratory-established target values. See the most current edition of NCCLS document H20–*Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods*, for a reference differential count method and the statistical methods for evaluating analyzer performance.

7.3 Evaluation of Proficiency Testing Results

Proficiency testing or external quality assessment programs provide laboratories with independent methods for evaluating test method accuracy. Such programs are used by regulatory agencies for laboratory accreditation to justify reimbursement for services.

In addition, the proposed system provides data for laboratory practice indicators which can be used to document laboratory performance as well as improvement in laboratory testing.

The following tables are useful for the evaluation of proficiency testing results, estimates of the magnitude of any discrepant results, and appropriate actions based upon those results.

Table 5. Score to be Assigned for Levels of Proficiency

| Score | Proficiency |
|-------|---|
| 0 | Result not scored due to unexpected problems with proficiency testing materials or their evaluation. |
| 1 | Result less than ± 1 SD of participant mean. |
| 2 | Result greater than ± 1 SD but less than ± 2 SD of participant mean. |
| 3 | Result greater than ± 2 SD but less than ± 3 SD of participant mean. |
| 4 | Result greater than ± 3 SD of participant mean but within the decision value limits given in Table 8. |
| 5 | Result within the decision value limits when the participant mean is below or above these limits (false negative); or outside the decision value limits when the participant mean is below or above these limits (false positive). See Table 8. |

Table 6. Laboratory Performance Interpreted from Proficiency Test Scores

| Score | Performance |
|-------|--|
| 1 | Good performance. |
| 2 | Acceptable performance. |
| 3 | Statistically expected variability for quantitative results in 5% of cases, i.e., greater than ± 2 SD of results |
| 4 | Significant technical problems (or clerical errors) with minimal potential for clinical misinterpretation. |
| 5 | Unacceptable laboratory practice leading to significant problems in the interpretation of laboratory results. |

Table 7. Actions to be Taken in Response to Proficiency Test Scores

| Score | Response |
|-------|---|
| 0-2 | No response required. |
| 3 | Careful monitoring of longitudinal proficiency testing results should be done to determine if there may be persistent biases in calibration of instrumentation, continuing instrumental problems, or other problems. |
| 4-5 | Require careful evaluation with appropriate responses, since such scores may indicate significant clinical and/or clerical problems. Actions could include instrument recalibration (or possibly replacement), improved data management, laboratory worker education, or other responses. |

Table 8. Decision Value Limits for Proficiency Test Evaluation

| Analyze | Units | Lower Decision Value | Upper Decision Value |
|----------|--------------|----------------------|----------------------|
| WBC | 10^9 /L | 3.0 | 12.0 |
| RBC | 10^{12} /L | 4.4♂ 3.9♀ | 6.2♂ 5.2♀ |
| Hb | g/L | 120♂ 110♀ | 180♂ 160♀ |
| Hct | L/L | 0.39 ♂ 0.30♀ | 0.54 ♂ 0.48♀ |
| MCV | fL | 80 | 100 |
| Platelet | 10^9 /L | 50 | 800 |

8 Assigning Values to Calibrators

The process has four stages:

- Reference values are conferred on fresh whole blood specimens.⁵
- Multichannel analyzer(s) are calibrated by the reference-assayed specimens.
- The multichannel analyzer(s) assay the candidate calibrator to assign values.

- The calibrator values are independently verified.

8.1 Methods to Confer Reference Values to Blood Specimens

CBC analyte values are assigned to fresh blood specimens (referred to as “reference specimens”) from healthy donors, judged by criteria in NCCLS document C28– *How to Define and Determine Reference Intervals in the Clinical Laboratory*, by methods that are presumed free of bias by meticulous conformance to the following principles. For the purpose of this document these values will be called *reference values*:

- 8.1.1 There shall be independent proof of analyte stability under the chosen conditions of specimen collection, anticoagulation and storage.
- 8.1.2 Total white cells (WBC) are defined as particles remaining within the volume range of 20 to 400 fL after lysis of red cells (RBC) by a reagent that removes red cell stroma residues within that size range.
- 8.1.3 Platelet (Plt) measurement is not included in this section.
- 8.1.4 Preanalytic processing, such as dilution and mixing, shall be carried out in containers and with volumetric devices that are shown (validated) to have no deleterious effect on the analytes and do not to contribute analytical interferences (e.g., adventitious particle and elutable solutes).
- 8.1.5 All volumetric devices shall be calibrated to internationally standardized measurements of mass and traceable to NIST certification or other equivalent valid standard of accuracy. Recalibration intervals shall be established by actual observations.
- 8.1.6 Hemoglobinometry⁸ and packed cell volume (PCV) measurement shall be performed as described in NCCLS documents H15–*Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood* and H7–*Procedure for Determining Packed Cell Volume by the Micro hematocrit Method*, respectively.
- 8.1.7 Wavelength and absorbance scales of the spectrophotometer used for hemoglobinometry shall be validated for every batch of measurements with filters that have been certified at least every two years by NIST or equivalent body.⁹
- 8.1.8 Measurements of length, such as PCV, shall be made with devices that have validated accuracy traceable to NIST or equivalent body.⁹
- 8.1.9 Cell-counting devices shall not, of themselves, be calibratable and shall conform to the following specifications:
 - Cells uniformly suspended in a noninterfering diluent will be caused to flow through a sensing zone by positive displacement.
 - The sensing device and preanalytic processes (e.g., red cell lysis) shall be validated for the ability to discriminate cell types.
 - The claimed volumetric accuracy of the volume-displacing device shall be validated.
 - The liquid path connecting the sensing zone and the displacing device shall be inelastic.
 - The liquid between the sensing zone and displacing device shall be freed of dissolved gas and cells or debris before each count.

- Each cell shall trigger the counting subsystem once and only once. Proof of conformance to this requirement shall be a part of the validation record. Some form of counting rate monitor will be useful for this purpose.
- The frequency of the simultaneous presence of more than one cell in the sensing zone, i.e., coincident cell error, shall be measured and arithmetically corrected within stipulated, validated limits.
- Means to control and monitor sensing thresholds shall be verified by reference materials, such as NIST or equivalent certified latex suspensions.
- Less than 0.25% of each count shall be contributed by nonspecific particles.
- Less than 0.5% of the counted particles shall reside at the lower or upper count trigger threshold settings.
- The time during which the measured cell suspension passes through the sensing zone shall be standardized and monitored with a precision of 0.1%. A change of this time outside this limit will invalidate the count.

8.2 Calibrate Analyzers

The assayed reference specimens will be presented to one or more automated hematology analyzers (assignment analyzers) of the type(s) for which the calibrator will be labeled. The preferred number of reference specimens and the number of replicate assays performed on each will be discussed below.

- 8.2.1** The mean of the values given by assignment analyzers to the reference specimens is divided by the mean of the reference values to obtain a calibration factor. If the assignment analyzer has automatic set point adjustment, further analyses will be assumed to be free of calibration bias. If the analyzer requires manual set point adjustment, the calibration factor should be calculated by hand and applied to the analyzer output off-line (the so-called "paper calibration" method). This will reduce or remove the human skill factor that is inherent in such adjustments.

Since various controllable physical variables may contribute to the analyzer's precalibration set point, it is prudent for these variables to be adjusted to the manufacturer's recommended nominal values. If the calibration procedure changes the nominal set point by more than 10%, an instrument malfunction should be suspected.

- 8.2.2** The minimum number of reference specimens and the degree of assay replication is determined by the method of limiting means. The cumulative mean of the differences between the reference values and the assignment analyzer values is plotted as a function of specimen sequence number. The resulting curve, describing the relationship between the two methods, will progressively change from being jagged to being smooth and asymptotic. The difference at this point expresses a ratio of estimate to parameter¹⁰ that will not change with further iteration. This ratio will be used as the calibration factor.

8.3 Assign Calibrator Values

The calibrator is assayed by the calibrated Assignment Analyzers. The mean of replicated assays will be the assigned values. The analyzer SD divided by the square root of the number of iterations will determine the standard error of this mean (SEM). Thus, 16 iterations will provide a standard error of 0.25 SD. The chosen number of iterations shall be validated for each type of assignment analyzer. Appropriate measures shall be used to detect drift or loss of precision during this run. The discovery of either of these abnormal conditions will invalidate the assignment of calibrator values.

8.4 Independent Verification

There should be provision for independent verification of the calibrator, whereby clinical blood specimens are assayed by current-lot calibrated instrument(s) in parallel with one or more prior calibrator lots and by properly validated reference or comparison methods. The values recovered by these calibrated instruments should encompass the reportable range of the method and should fall within the combined confidence limits of the calibrator, the calibration process error, and the imprecision of the analyzers.

8.5 Process Validation

No process or part of a process used for the assignment of values should be deemed suitable for its intended purpose, unless its claimed suitability is based on documented proof obtained under worst-case conditions. Whenever possible, the design of validation tests should be based on absolute units of mass, length, volume, etc., traceable to national or international standards.

Software, provided by a manufacturer as a component of a measuring device or used for process control or data management, should have been validated according to national (e.g., FDA) or international requirement governing the device distribution.

Manufacturer's specifications for devices used in the assignment process should not be accepted at face value but should be verified by independent measurement. For example, the swing radius, spinning time, angular velocity, and temperature rise of centrifuges should be verified by measuring devices that have been calibrated against NIST or equivalent standards.

Procedural descriptions of validation tests must include a revalidation schedule, if there is any possibility that the process might change with time or repeated use. The decision to dispense with revalidation must, of itself, be validated.

Appendix. t Factors to Convert Standard Deviation to 95% Confidence Limits as a Function of n

| No. of assays (n) | t for 95% limits | No. of assays (n) | t for 95% limits |
|-----------------------|--------------------|-----------------------|--------------------|
| 1 | 12.7062 | 17 | 2.1098 |
| 2 | 4.3027 | 18 | 2.1009 |
| 3 | 3.1824 | 19 | 2.0930 |
| 4 | 2.7764 | 20 | 2.086 |
| 5 | 2.5706 | 21 | 2.0796 |
| 6 | 2.4469 | 22 | 2.0739 |
| 7 | 2.3646 | 23 | 2.0687 |
| 8 | 2.306 | 24 | 2.0639 |
| 9 | 2.2622 | 25 | 2.0595 |
| 10 | 2.2281 | 26 | 2.0555 |
| 11 | 2.2010 | 27 | 2.0518 |
| 12 | 2.1788 | 28 | 2.0484 |
| 13 | 2.1604 | 29 | 2.0542 |
| 14 | 2.1448 | 30 | 2.0423 |
| 15 | 2.1315 | 31 | 2.0395 |
| 16 | 2.1199 | 32 | 2.0369 |

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Example: Calculate set point confidence limits

Given a calibrator with RBC = $4.65 \times 10^{12}/L$

Claimed instrument imprecision CV% = 0.8

Mean recovered calibrator value for $n = 10$ is $4.67 \times 10^{12}/L$

t for $(n - 1)$ in the Appendix = 2.2622

Is the recovered value acceptable?

See proof below

Convert CV% to CV

$$CV = (CV\% \div 100) = 0.008$$

Convert CV to CV at 95% confidence

$$CV_{95\%} = CV \times t = 0.008 \times 2.2622 = 0.0181$$

Convert $CV_{95\%}$ to CV_{SEM} (Standard error of the mean)

$$CV_{SEM} = CV_{95\%} \div \sqrt{10} = 0.0181 \div 3.1622 = 0.0057$$

Convert CV_{SEM} to calibration factor (CF)

$$\text{Lower acceptable CF} = 1.0000 - 0.0057 = 0.9943$$

$$\text{Upper acceptable CF} = 1.0000 + 0.0057 = 1.0057$$

Convert CF to RBC values

$$\text{Lower acceptable RBC value} = \text{Assigned calibrator value} \times \text{lower CF}$$

$$\text{Lower acceptable RBC value} = 4.65 \times 0.9943 = 4.6235$$

$$\text{Upper acceptable RBC value} = \text{Assigned calibrator value} \times \text{upper CF}$$

$$\text{Upper acceptable RBC value} = 4.65 \times 1.0057 = 4.6765$$

Acceptable calibrator recovery range (rounded to two decimal places)

$$4.62 - 4.68 \times 10^{12}/L$$

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Related NCCLS Publications*

- C24-A** **Statistical Quality Control for Quantitative Measurements: Principles and Definitions Second Edition; Approved Guideline (1998).** This guideline describes the purpose of internal quality control; defines various analytical intervals such as “analytical run”; and addresses the use of quality control material and control data, including the use of data in quality assurance and interpretation.
- C28-A** **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (1995).** This document provides guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- EP5-A** **Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1998).** This document provides guidelines for designing an experiment to evaluate the precision performance of clinical chemistry devices; recommendations on comparing the resulting precision performance claims and determining when such comparisons are valid; and manufacturer’s guidelines for establishing claims.
- GP2-A3** **Clinical Laboratory Technical Procedure Manuals-Third Edition; Approved Guideline (1996).** This document provides guidelines that address the design, preparation, maintenance, and use of technical procedure manuals, in paper or electronic formats, for use by the patient-testing community.
- GP19-A** **Laboratory Instruments and Data Management Systems: Design of Software User Interfaces and End-User Software Systems Validation, Operation, and Monitoring; Approved Guideline (1995).** This guideline identifies important factors that designers and laboratory managers should consider when developing new software-driven systems and selecting software user interfaces. Also included are simple rules to help prepare validation protocols for assessing the functionality and dependability of software.
- GP21-A** **Training Verification for Laboratory Personnel; Approved Guideline (1995).** This guideline provides background and recommends an infrastructure for developing a training verification program that meets quality/regulatory objectives.
- H7-A2** **Procedure for Determining Packed Cell Volume by the Micro hematocrit Method-Second Edition; Approved Standard (1993).** *American National Standard.* This document describes the standard Micro hematocrit method for determining packed cell volume, including recommended materials and information on potential sources of error.
- H15-A2** **Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood Second Edition; Approved Standard (1994).** *American National Standard.* This document describes the principle, materials, and procedure for reference and standardized hemoglobin determinations. It includes specifications for secondary hemoglobin cyanide (HiCN) standards.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

Related NCCLS Publications (Continued)

- H20-A** **Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard (1992).** This standard describes automated differential counters and establishes a reference method, based on the visual (or manual) differential count for leukocyte differential counting, to which an automated or manual test method can be compared.
- H26-A** **Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers; Approved Standard (1996).** This standard establishes performance goals for analytical accuracy and precision based on mathematical models for the following measurements: hemoglobin concentration, erythrocyte count, leukocyte count, platelet count, and mean corpuscular volume.
- H44-A** **Methods for Reticulocyte Counting (Flow Cytometry and Supravital Dyes); Approved Guideline (1997).** This document provides guidance for the performance of reticulocyte counting by flow cytometry. It includes methods for determining the accuracy and precision of the reticulocyte flow cytometry instrument and a recommended reference procedure.
- M29-A** **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.
- NRSCL8-A** **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document contains proposed definitions for use in NCCLS standards and guidelines, and for use in submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

NOTES

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