ASVCP Guidelines: Allowable Total Error Hematology
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1 Introduction

Analytical quality requirements (a.k.a. quality specifications) are pre-determined benchmarks used to judge analytical performance of laboratory instruments or methods.[1] Quality requirements may vary by type of laboratory, measurand concentration (e.g., low vs. high) and species tested. Quality requirements can be derived from several sources, including governmental regulatory requirements, expert opinion, biological variation data, and current instrument performance. Regardless of source, it is essential that quality requirements be clinically relevant for medically important measurand concentrations and be realistic for available laboratory technology.[2]

Allowable total error (TEa) defines a quantitative goal combining imprecision (random error) and inaccuracy (systematic error, or bias) to determine acceptable variation in a single measurement procedure without interfering with the clinical interpretation of patient data.

2 Scope

Objectives of this guideline are to provide TEa recommendations for hematology measurands routinely assayed in veterinary practice and to provide an overview of how these recommendations were derived. A worksheet for determining whether instrument performance meets these recommendations is also included. Similar to biochemistry testing, TEa recommendations for hematology testing in veterinary medicine are needed to facilitate instrument performance evaluation, method comparison, and quality control validation. Intended audiences include, but are not limited to, reference laboratories, in-clinic laboratories, and animal health diagnostic companies supplying hematology instruments to the veterinary market. TEa is proposed here for those measurands in common clinical use and likely to be evaluated with routine quality control procedures. Furthermore, the TEa recommendations were based on clinician input regarding dogs, cats, and horses, along with data generated from quality control material (QCM) and dog specimens using automated methods. This guideline is thus not all-inclusive but presents TEa recommendations considered to be suitable for the current instruments and methods commonly used for veterinary hematology measurands. While blood from non-mammalian species was not tested, the recommendations could serve as a baseline for laboratories evaluating blood from non-mammalian animals.

3 Abbreviations and definitions

Accuracy – Closeness of agreement between the results of a measurement and the true concentration of the measurand. Accuracy is the opposite of inaccuracy or bias.[3]

ASVCP –American Society for Veterinary Clinical Pathology

Bias (a.k.a. inaccuracy) – Total systematic error, which includes constant and proportional bias. Bias is the difference between the test instrument's measured result and the true value (e.g., as measured by a reference method or as defined by a known standard). The term bias in difference

plot analysis (expressed in measurand units) equals the difference between the values of the two methods being compared or the average of all the differences between the paired sample values. Bias may also be expressed as a percentage according to the formula:

$$Bias\% = \frac{Mean_{target} - Mean_{measured}}{Mean_{target}} \times 100$$

Bias, constant – When the degree of systematic error remains the same over the range of measurand concentrations (i.e., results of one method are consistently above or below another method and roughly by the same amount, regardless of measurand concentration).[3]

Bias, proportional – When the magnitude of systematic error changes as the measurand concentration changes.[3]

Biological variation – Expected physiologic fluctuations of a measurand, which can include variation within an individual or between individuals.[4]

CLIA –Clinical Laboratory Improvement Amendments.

CV (coefficient of variation) – A measure of imprecision (random error), biologic variation, or other variability in a population; mathematically, CV is standard deviation (SD) divided by the mean and expressed as a percentage.[3]

Comparability Testing – A quality assurance procedure in which measurement results from two or more instruments or methods are compared to each other for purposes of analytical performance assessment. Comparability testing can be a component of formal EQA/PT programs or can be carried out independently within a laboratory or network of laboratories.[5] Total allowable error is one tool that is used in comparability testing and aids in assessment of whether results from different instruments can be used interchangeably without causing clinical error.[3]

Decision Threshold – Clinical or medical decision limit (i.e., numerical value) at which important clinical decisions regarding testing or treatment are made for a particular measurand.

External Quality Assessment (external quality assurance (EQA) or proficiency testing (PT)) – Interlaboratory comparisons and other performance evaluations that determine total testing performance and may extend throughout all phases of the testing cycle, including interpretation of results. These include peer interlaboratory comparisons in which multiple laboratories measure the same specimen using the same test methods, and in some cases the same reagents and controls, as well as comparison with known values of reference materials.[6]

EQA/PT specimen, testing item, test material, or check specimen panel - A specimen containing measurands of undisclosed concentrations or compositions sent to a participating laboratory to assess the laboratory's testing competency.[6]

Imprecision (random error or random variation) – Lack of agreement between independent, repeated results obtained from the same specimen under specified conditions.[7] It is represented by the standard deviation (in units of the test) or coefficient of variation (in units of percent).[3]

Instrument performance study – A study performed to characterize an instrument's analytical performance capability, represented by bias (inaccuracy) and imprecision (random error). Data from an instrument performance study can be used to calculate observed total error (TEobs).[3]

Mean – Average of values measured, Mean = $(x_1 + x_2 + \ldots + x_n)/n$

Measurand - A particular quantity subject to measurement under specified conditions (e.g. the enzymatic activity of alkaline phosphatase at 37°C).[6]

Peer group – Used for comparison of quality requirements and defined by the same instrument and/or method as that used by the participating laboratory or testing site.

Precision – Closeness of agreement between independent, repeated results obtained from the same specimen under specific conditions. These may be derived in the same day (repeatability study) or on different days (within laboratory precision).[7] Note: The definition of precision has become more complex in recent years and is frequently being modified. Readers are referred to other sources for further definition.[8]

Proficiency testing (PT) – One measure of laboratory competence derived by means of an interlaboratory comparison; implied in this definition is that participating laboratories are using the same test methods, reagents and controls. PT is often used synonymously with EQA but may specifically refer to testing performed in compliance with state or federal regulations.[9]

Reference Interval – An interval that contains all the possible values between and including an upper and lower limit. Reference limits are defined such that the reference interval contains a specified proportion of values from a well-defined, typically clinically healthy reference population. Reference interval is preferred over the term reference range.[10]

Repeatability – Precision of analysis when repeated using the same operator, measurement procedure, equipment, time, and laboratory.[7]

Repeat patient testing (RPT) – Repeat testing of individual patient specimens under specified conditions that is used as a statistical quality control method. RPT exploits the fact that specimen deterioration under defined conditions (fixed time interval, storage conditions, etc.) causes an expected degree of variation in results; any variation in results exceeding this threshold may indicate a problem with the test system.[11,12]

QA (quality assurance or assessment) – Laboratory procedures that monitor and improve laboratory performance and seek to minimize pre‑analytical, analytical, and post‑analytical error. QA includes many nonstatistical procedures (e.g. personnel training, use of standard operating procedures, etc.)[13]

QALS (Quality Assurance and Laboratory Standards Committee of the ASVCP) – The ASVCP committee charged with encouraging and promoting the establishment of standards for the performance of laboratory procedures on animal specimens.

QC (quality control) – Procedures that monitor analytical performance of instruments and detect analytical error.[13]

QCM (quality control material) – A test material intended by its manufacturer to be used for QC of laboratory testing. Measurement of QCM monitors the entire test system (operator, reagents, and instrument analytical function). QCM may be used to carry out an instrument performance study or to monitor routine analytical performance. "Assayed QCM" is QCM that has been measured by the manufacturer, which then provides target means, ranges, standard deviation and CV for that QCM for specific instruments or methods.[13]

Quality Goal Index – A numerical index that reflects whether imprecision, bias, or both are contributing to an observed analytical error. QGI may be calculated according to the formula[14]

$$QGI = \frac{\%Bias}{CV}$$

Quality Requirement (quality specification) – A benchmark to which the analytical performance of a laboratory instrument is compared. Also see allowable total error (TEa).[3]

Standard Deviation (SD) – A measure of variability or diversity associated with random error or imprecision. SD demonstrates the variation or dispersion from the mean (average or other expected value) during repeated measures. A small SD indicates that data points tend to be very close to the mean, whereas a large SD indicates that data points are spread over a wide range of values. SD is the square root of a dataset's variance.[3]

$$s = \sqrt{\frac{\sum(x_i - \overline{x})^2}{(n-1)}}$$

TE (total error, total analytical error) – The sum of random error (imprecision) and systematic error (bias or inaccuracy). This term may also incorporate other sources of error (e.g., pre-analytical variation, biologic variation, and other factors) that contribute to the variation seen in patient results. TE may be expressed in measurand units or as a percentage.[3]

TEa (allowable or desirable total error) – A quality requirement that sets a limit for combined imprecision (random error) and bias (inaccuracy, or systematic error) that are tolerable in a single measurement or single test result to ensure clinical usefulness.[3] Recommendations for hematology TEa are found in section 5.

TEobs (observed or calculated total error) – The sum of measured random error (imprecision) and systematic error (bias/inaccuracy) that can be calculated from instrument performance data according to the formula as defined in this guideline

$$\textbf{TEobs = 2CV + bias(\%) \underline{or} 2SD + bias (measurand units)}$$

 TEobs must be calculated for each measurand, is unique to an individual instrument/method, and may vary with measurand concentration or activity. Absolute values for bias should be used in these formulae. The constant 2 is rounded from the bi-directional Z score of 1.96 (standard normal deviate).[15]

Value (of a quantity) – Magnitude of a particular quantity generally expressed as a unit of measurement, which may be multiplied by a number (e.g. 3.5×10^{3} cells/μL, 5 U/L).[11] It can be used synonymously with result.[16]

Variable – A quantity of interest, whose value or magnitude fluctuates or changes (e.g. creatinine).[17]

Z score – A unitless number that is a coefficient indicating the number of standard deviations from the mean. The z score (a.k.a. standard score, z value) is arbitrary in TEa and dependent upon the stringency desired for the test. The ASVCP consensus-approved TEa guidelines and CLIA documentation assign the z score of 2 for calculation of TEa in laboratory medicine.[18]

4 Allowable total error (TEa)

4.1 Introduction to TEa

The concept of total error was first introduced in 1974.[18,19] Analytical imprecision (random error) and bias (systematic error) were combined into a single measure representing the uncertainty of a test result. The ideal situation is to have a highly accurate and precise measurement, i.e. low bias and low coefficient of variation (CV) or standard deviation (SD), respectively.

A commonly used quality requirement derived from the total error concept is allowable total error (TEa), which is derived from the amount of allowable variation around medically important measurand concentrations or decision thresholds. This quality requirement has been found to be cost-effective in human medicine because stringency of quality control (QC) is specified by the accuracy and precision needed to make clinical decisions rather than the highest accuracy and precision possible. TEa expresses the largest amount of error (combined imprecision and bias) that is tolerable for a given laboratory measurement (i.e., the maximum allowable error that does not interfere with medical interpretation of the patient's test result). As with TEa for veterinary biochemistry testing, TEa for hematology measurands (Table 1 and Table 2) was established by the Quality Assurance and Laboratory Standards (QALS) committee based on a combination of clinical decision-making thresholds and current reference laboratory instrument performance, as detailed in Appendix A. TEa can be used when assessing instrument performance.

There are currently several manuscripts that report the use of TEa in veterinary laboratory medicine, as well as a review article outlining the use of TEa for in-clinic instrument performance evaluation.[20-26] Additionally, the American Society for Veterinary Clinical Pathology (ASVCP) published TEa recommendations for biochemistry testing in veterinary medicine.[3] TEa can be used to aid instrument selection if manufacturer's claims for instrument performance are available or if several instruments are being evaluated. TEa can also be compared to an instrument's calculated or observed total error (TEobs) to help determine whether that instrument's analytical performance is acceptable. If analytical performance is deemed acceptable, TEa can further be used during quality control (QC) validation of that instrument. Finally, TEa can be used to guide comparison of test results across laboratories and clinics using the same or different analytical methods. For example, TEa can be used to help interpret results from external quality assurance or proficiency testing (EQA/PT) programs or to help interpret results of comparability testing, where a reference laboratory is used to check in-clinic or other laboratory results.[27] Additional information about TEa can be found in CLSI- C54-A.[28] Factors impacting TEa include species, measurand concentration, clinical use of patient results, and type of laboratory. In other words, TEa for the same measurand may vary considering these factors, with examples listed below:

Species	Dogs, cats, horses, birds, etc.

Measurand concentration	High, within reference interval, and low measurand concentrations
Clinical use of patient results	Different medical decision thresholds
Laboratory	Reference laboratories, veterinary practices, toxicology laboratories, and other industry laboratories, all of which may serve different species and patient populations and may use different analytical methods.

While the above factors are all recognized to influence TEa, the recommendations proposed in this guideline were largely based on clinician recommendations for dogs, cats, and horses as well as instrument performance based on quality control material (QCM) and dog specimens.

4.2 Special considerations when generating hematology TEa
Below are considerations specific to hematology TEa

4.2.1 Quality control material (QCM)
The QCM standardly used for hematology instruments are optimized for the analysis of human specimens. Companion animal, species-specific, commercially available QCM is not known to exist, and TEobs may vary for different measurands in the various species. In particular, it would be expected that TEobs using human QCM would be lower than that based on species-specific material, as seen in a recent study.[25] Additionally, not every CBC measurand has a target value provided by the manufacturer (e.g., MCHC). Therefore, use of whole blood from a species of interest is required for complete evaluation of a hematology instrument. A new concept, repeat patient testing (RPT), may overcome some of the disadvantages of commercial quality control materials, but has not yet been investigated thoroughly for use in veterinary laboratories.[12,29] Use of patient specimens for quality control provides a matrix of veterinary origin, and specimens can be manipulated to produce results that fall at or close to decision thresholds and provide a less expensive option compared with commercially available QCM (see section 4.3.1.1).

4.2.2 WBC differential
The WBC differential may be generated using automated techniques, manual techniques, or a combination of the two. Making TEa recommendations for WBC differential counting is challenging due to differences in analytical performance of reference laboratory and in-clinic instruments and the inherent high imprecision of manual differential counting (in cases where automated differential counts are inaccurate due to morphologic alterations and a manual count is reported).[25] The WBC differential (whether automated or manual) is likely to demonstrate higher TEobs than other

hematology measurands, particularly for enumeration of eosinophils and monocytes.[25] TEa recommendations for neutrophils and lymphocytes (Table 2) should be achievable by point of care hematology analyzers, although Cook et al found performance of several analyzers to be highly variable.[30] For any instrument (reference laboratory or in-clinic laboratory), before an automated differential is accepted for any patient, review of a good quality blood smear is recommended to verify automated findings.[31] If discrepancies are noted, or any abnormalities in WBC morphology known to interfere with or limit automated WBC enumeration are observed, clarity of the automated differential cytogram as well as the quality of the film preparation should be assessed. If the automated differential is deemed possibly inaccurate, a manual differential should be performed and reported, realizing that manual differentials are associated with high imprecision based on the number of leukocytes standardly counted in veterinary laboratories.[32] In general, TEa recommendations given here are intended for automated testing; however, these recommendations can also be applied to manual WBC enumeration by laboratories routinely using manual counts. If TEobs exceeds TEa, misdiagnosis could result. Maintenance, troubleshooting and manual blood smear review should all be routinely performed.

4.2.3 Platelets

Blood specimens from cats are known to clot quickly, and their platelets are frequently clumped upon evaluation of a blood smear. Therefore, TEobs in cats can be much more variable than in other species, and high TEobs for feline platelets was found due to imprecision in one study.[25] Therefore, TEa for platelets presented in this guideline focus on dogs and horses. Platelet concentrations <50,000/µL in any species are often associated with a higher degree of error than the recommended TEa of 20%, even when TEobs is based only on imprecision (2*CV). Imprecision is particularly high when counts are extremely low (e.g., <10,000/µL). However, at these extremely low concentrations, high error means that small changes are not biologically significant despite the fact that clinical decisions are often made based on such changes. For example, a 50% TEobs for 10,000/µL platelets means that the patient result can range from 5,000/µL to 15,000/µL.

4.2.4 Reticulocytes

Automated reticulocyte counts in cats are problematic due to the presence of both punctate and aggregate reticulocytes, and higher CV is observed for reticulocytes in cats than in dogs.[26] Reticulocyte counts in horses are rarely clinically relevant and may be subject to increased error, given the higher degree of error associated with reticulocyte counts <60,000/µL (see Appendix). While some data support a relatively close correlation between manual and automated reticulocyte counts, precision of manual reticulocyte counts is thought to have higher error.[33,34] Therefore, reticulocyte recommendations in this document focus on reticulocyte numbers generated by

automated methods in dogs. However, in general, improvements in instrument performance as well as better-defined decision thresholds are needed for reticulocyte interpretation.

4.3 Instrument performance evaluation using TEa

Detailed instrument performance evaluation and validation procedures are commonly used in reference laboratories, where determination of bias and CV should be carried out within recommended ASVCP guidelines and at the discretion of the quality control specialist. Periodic assessment of TEobs for EQA/PT should be performed on a quarterly basis.[13] Indications for evaluating instrument analytical performance include:

1. A new instrument is being considered for purchase
2. During instrument performance verification (when a new instrument is evaluated to ensure that it performs according to manufacturer's claims) or evaluation
3. Exploring unacceptable EQA/PT results
4. Fulfilling periodic TEobs assessments

For purposes of calculating the total error of a measurand using a particular method or instrument (TEobs), Westgard originally used TE = bias(%) + 1.65CV.[35] This formula is the basis for the TEobs calculation used in this document, which is currently the most widely accepted formula:[36]

$$TE_{obs} = absolute\ bias\% + 2CV$$

If units of the measurand are used, then the equation used to calculate an instrument's total error (TEobs) changes to:

$$TE_{obs} = absolute\ bias\ (in\ measurand\ units) + 2SD$$

where SD is standard deviation.

Absolute values for bias should be used in these formulae (i.e., negative values should not be used).[15]

Calculation of an instrument's TEobs (for purposes of comparing to TEa) can be based on routine daily QC data and/or periodic EQA/PT data, both of which are recommended to ensure ongoing production of reliable laboratory results. The frequency of quality assurance (QA) monitoring can be determined by the QC specialist based on the number of samples analyzed per day, known inherent drift of the analytical method, perception of previous problems noted in the laboratory, cost of reagents, and other factors.[3]

4.3.1 Quality Control Material (QCM)

Analysis of QCM is done to regularly assess instrument performance and can also be conveniently used for calculating TEobs. Commercially available, stable, assayed QCM is most commonly used and may be purchased from a number of companies (See Appendix B). The choice of QCM depends on laboratory preference regarding commutability across instruments/methods, the number, type and concentration of

measurands present within the materials, shelf-life/stability, cost, etc. In addition, both in-clinic QC and EQA/PT require different types (concentrations, species, cell sizes, etc.) of QCM, dependent upon the instrument, reference intervals, expected changes due to disease, and species evaluated at the facility. Hematologic QCM must be selected based on the methodology of the instrument; QCM for impedance-based and flow cytometric-based instruments are not interchangeable. QCM from different lots may not have the exact same measurand concentrations, which must be factored into the control limits used to decide if QC data are in-control or out-of-control.[37] Ideally, a minimum of two levels (measurand concentrations) of assayed QCM should be used to determine instrument performance.[22] The concentration of measurand in the QCM should be at or near those of decision thresholds and/or reference limits. If only one concentration of QCM is used, then ideally it should be consistent with reference values for the species.

All commercially available QCM and calibration materials have a lot number and expiration date based on proper storage of unopened vials. QCM should be labeled with the date it is opened and the expiration date based on opening. All QCM should be promptly discarded upon reaching either of its expiration dates (i.e., expired QCM should never be used). Hematologic QCM may degrade more quickly upon opening than QCM for biochemical testing, and it may show signs of degradation near the end of (although prior to) its expected shelf life.

Manufacturer's recommendations regarding storage and handling should be followed and included in the laboratory's Standard Operating Procedures.[38] QCM that are transported or stored under inappropriate conditions may lead to errors. For example, using compromised QCM can trigger unnecessary troubleshooting and/or calibration. Furthermore, if compromised calibration materials are used to calibrate an instrument, it will lead to systematic error. Therefore, conditions known to alter the stability of the QCM (temperature, light, humidity, length of storage, etc.) must be monitored to ensure its stability. If it is suspected that the stability of the QCM is compromised, it should be discarded and replaced. Shipment of hematology QCM and whole blood between laboratories frequently results in disparate measurements due to transport conditions and degradation over time. Even under appropriate conditions of transport and storage, measurand results may vary over the lifetime of the QCM while remaining within the expected intervals. Therefore, when comparability assessment between laboratories is desired, assayed QCM should be analyzed at approximately the same time (i.e., within 6 hours), on the instruments to be compared. This may require splitting and shipping of QCM in aliquots instead of analyzing the QCM on different days at different facilities. Timing and arrival should be planned so that weekends and holidays are avoided. In contrast, QCM for chemistry and endocrinology can usually be aliquoted and frozen for some time.

4.3.1.1 Use of patient specimens

Because of the instability of cells in anticoagulated blood, commercially available, assayed QCM typically contains either synthetic components or stabilized cells that might not behave in exactly the same manner as patient specimens. However, because it is more stable than patient specimens, assayed QCM is preferred to assess reagent or instrument drift. While assayed, commercially available, hematology QCM may include bovine, porcine, equine and other species' cells, a companion animal-specific assayed hematology QCM currently does not exist. While commercially available QCM is adequate to assess the instrument, it may not be adequate to assess species-specific differences in observed error. Therefore, use of patient whole blood specimens is still important in species-specific assessment of total error in veterinary medicine. For this purpose, whole blood may be used to generate species-specific, same-day precision using a minimum of 5 replicates.[13] Whole blood should not be maintained for quality assessment for more than 24 hours, as specimens can denature and result in erroneous values.[39]

Patient specimens can also be manipulated by diluting or concentrating the sample to obtain hematology measurand concentrations below, within, and above their reference interval.[40] These samples can then be used to determine TEobs at clinically relevant concentrations, with bias determined by comparing with a reference laboratory. While this may provide a less expensive option than QCM for instrument performance evaluations and comparative testing at important decision thresholds, it requires a defined protocol with trained personnel. It can also be time-consuming.

4.3.2 Assessing Imprecision

The conditions under which any precision study is conducted should be documented in writing (e.g., date, operator, instrument, and specimen information). A minimum evaluation, performed using two measurand concentrations, may be obtained from measuring a stable QCM five times within the same day or over 5 days. If using patient samples or sample pools, measurements should be performed within the same day. These data are then used to calculate mean, SD, and CV.[22] For guidance concerning more sophisticated precision evaluation (e.g., for user verification of manufacturer's precision claims), readers are referred to other resources.[7] Precision evaluation should be performed at measurand concentrations of medical interest, especially if decision thresholds are known.

4.3.3 Assessing Bias

Assessment of bias is relative. In clinical pathology laboratories, best practice dictates that target means for calculation of bias be based on data from a true reference

method ("definitive" method). However, comparison with reference methods of analysis may be expensive; therefore, other comparative standards are typically used, including target means established by the manufacturer for QCM, results from comparative testing with laboratories with known expertise in analysis of the measurand being evaluated, or peer group means in external quality assurance programs.[3] While a full discussion of the advantages and limitations of different ways to determine bias are beyond the scope of this guideline, two commonly used and recommended methods to determine bias in the clinical setting are summarized below. Whether one or both methods are chosen for bias determination by a particular laboratory will depend on many factors, including the intended goals of the laboratory for determining bias. Readers are referred to other resources for additional discussion.[23]

1. Comparison with target values provided by manufacturers of assayed QCM. Assayed QCM may be repeatedly measured for at least 5 days to determine mean, bias, SD and coefficient of variation. In this situation, the mean of the results should be compared to the manufacturer's mean to determine bias as detailed in section 4.3.4. These data can then be used to calculate TEobs of a given measurand. The assayed QCM should be specific for the equipment and methods being evaluated; the instrument manufacturer should be consulted if there is any doubt regarding QCM suitability. This method is recommended for in-clinic analyzers, as there are currently few EQA/PT programs with peer groups that are large enough to provide adequate statistics for veterinary in-clinic instruments.

2. Comparison with peer group means through EQA/PT program participation. The difference between the participating laboratory's result and the peer group mean is used to represent bias. For additional detail concerning EQA/PT program selection and participation, readers are referred to the ASVCP's guideline on this topic.[27]

4.3.4 Instrument performance evaluation steps

        Determination of TEobs is described below. Additionally, a worksheet is provided in Appendix C to help guide users through the process of determining TEobs. All steps should be carried out by appropriately trained personnel who are knowledgeable regarding the analyzer's operation and the facility's quality assessment program. Calculations can easily be done using commercially available software programs. Calculations should be performed for each measurand and each QCM concentration. At least two different measurand concentrations (e.g., normal and high, or high and low) of QCM should be evaluated.

1. Measure each QCM daily at least five times.[22] Five repetitions in one day is possible but does not incorporate potential interday variation that mimics conditions when assessing samples from hospitalized patients over time. If

QCM is stable according to the manufacturer for at least 5 days, interday assessment is recommended. Using these data, for each QCM and each measurand, calculate

    a. Mean (average)
    b. Standard deviation (SD)
    c. Coefficient of variation (CV):

$$CV\ (\%) = \frac{SD}{Mean} \times 100$$

The mean, SD, and CV derived from these QC data are referred to as the 'measured,' 'calculated,' or 'observed' mean, SD, and CV.

2. Calculate the analyzer's measured bias using the measured mean and the QCM manufacturer's reported mean (i.e., target mean) for the assayed control material (using the same instrument and/or method as that used by the analyzer) according to the formula:

$$Bias\% = \frac{Mean_{target} - Mean_{measured}}{Mean_{target}} \times 100$$

QCM manufacturer's reported means are commonly found in the QCM package insert, categorized according to the instrument and method producing the assayed values. Measured bias may be a positive or a negative number, depending upon whether the analyzer's results are lower or higher than the manufacturer's reported mean. If bias is a negative number (e.g., − 5.0%), then the *absolute number* (5.0%) should be used in step 3, below.

3. Calculate the analyzer's TEobs, using measured CV and measured bias, according to the formula:

$$TE_{obs} = absolute\ bias\% + 2CV$$

4. Compare measured TEobs to recommended TEa found in Table 1. If TEobs ≤ TEa, then the quality requirement is met and the instrument is considered suitable for measurement of that measurand. If TEobs > TEa, then several options exist.

4.3.5 When TEobs is greater than TEa

    If calculated TEobs is greater than that which is considered acceptable (TEa), attempts should be made to identify and correct causes of imprecision (high CV) and/or

inaccuracy (high bias).[24] Use of special calculations, such as the Quality Goal Index may be helpful in determining if the poor performance is due to imprecision, inaccuracy, or a combination of both.[14] If unacceptably large imprecision is suspected, then a more rigorous precision study should be performed, including verification of manufacturer performance claims, if this has not already been done.[7] If unacceptably large bias is suspected, then the means by which bias was determined should be re-visited to determine if the targets are optimal. Instrument performance re-evaluation using a more appropriate target for bias determination (i.e., a different representation of true measurand concentration) could be considered.

　　　　If these sources of error cannot be corrected or if problems occur repeatedly, the manufacturer of the instrument and/or a board-certified clinical pathologist with expertise in QA should be called upon for further assessment. Further assessment may include attempts to improve performance capability by analyzer adjustments, operator training, reagent replacement with a new reagent or a product from a different manufacturer, or, potentially, analyzer replacement.[24]

　　　　Alternatively, the initial quality requirement may be relaxed. This approach is acceptable only if diagnostic judgment deems that additional analytical error can be tolerated. Furthermore, this option should only be used upon consultation with a board-certified veterinary clinical pathologist or other QC specialist. Relaxation of the TEa for a particular measurand requires education of ALL clinicians using analyzer results that this measurand is associated with larger error than is recommended. Use of a TEa higher than that recommended in this document should be justified and documented in a laboratory handbook.

5 TEa recommendations

　　　　Table 1 and Table 2 summarize TEa recommendations for hematologic measurands. These recommendations were partially based on instrument performance using QCM (see Appendix A for the control ranges evaluated). Each instrument's manufacturer guidelines should be consulted to determine the range of values supported by the instrument. Most instruments cannot accurately quantify extreme values. It is worth noting that use of quality requirements for hematology testing focuses on automated data, and manual review of blood smears is necessary to verify automated findings.

6 TEa vs. biological variation-based quality specifications

　　　　Study of biological variation (BV) is growing in veterinary clinical pathology, and online datasets are available.[41] Data from BV studies may be used to establish quality specifications for imprecision, bias, and total error.[42] For some measurands, BV-based quality requirements may be more stringent than what is achievable using current state-of-the-art instrumentation.[3] For such measurands, less stringent quality specifications (such as allowable total error based on decision thresholds) may be more realistic.

Biological variation of hematology measurands has been studied in dogs; example quality specifications calculated from selected data are presented in Table 3. Based on instrument performance data gathered by the authors (Appendix A), current veterinary reference laboratory state-of-the-art instrumentation can typically meet "minimum" BV-based TE. In fact, it can often meet "desirable" TE for most hematology measurands. The ability to meet "desirable" and "optimum" BV-based TE varied by measurand and institution. Unsurprisingly, "optimum" TE (the most stringent BV-based quality requirement) was met least often and is currently not recommended for routine assessment of hematology instrument performance. Overall, TEa values recommended in this guideline are comparable to published minimum TE based on biological variation data (Table 3), and either type of quality requirement can be used to evaluate hematology instrument performance.[43-45]

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8 Web resources

Glossary of QA terms, Westgard QC glossary:
http://www.westgard.com/glossary.htm

CLIA website:
https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html

Summary of CLIA recommendations:
https://www.westgard.com/clia.htm

American Society for Veterinary Clinical Pathology guideline documents:
http://www.asvcp.org/pubs/index.cfm

Biological variation:
http://vetbiologicalvariation.org

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11 Acknowledgments

12 Tables

Table 1. Allowable total error (TEa) for automated hematologic measurands, applicable for all concentrations (low/normal/high).[a] See Appendix A for derivation of TEa values.

Measurand	TEa	[b]CLIA Value
RBC	10%	6%
Hgb	10%	7%
Hct/PCV	10%	6%
MCV	7%	---
MCHC	10%	---
Reticulocytes[c]	20%[d]	---
WBC (**Reference laboratory**)	15%[e]	15%
WBC (***In-Clinic laboratory**)	20%	---
Platelets (**Reference laboratory**)	20%[f]	25%
Platelets (***In-Clinic laboratory**)	25%	---

CLIA = Clinical Laboratory Improvement Amendments; HCT = hematocrit; HGB = hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; PCV = packed cell volume (spun HCT); RBC = red blood cells; TEa = total allowable error; WBC = white blood cells

[a]Extreme values not supported by most instruments

[b]Clinical Laboratory Improvement Amendments (CLIA) is the accepted performance standard in human medicine in the United States and is therefore included here for comparison.[36]

[c]This recommendation is only applicable to canine absolute reticulocyte counts. Feline reticulocytes may be associated with higher error using automated methods.

[d]Reticulocytes <60,000/µL can be associated with high TEobs and are not considered clinically relevant. (See Appendix)

[e]Instruments were typically able to achieve an error (2*CV) <15% in patient samples with WBC counts as low as 1000 cells/µL.

[f]Instruments were typically able to achieve an error (2*CV) <20% in patient samples with platelet concentrations >50,000/µL. Concentrations below this number were frequently associated with a higher degree of imprecision (up to 50-75%).

*In-clinic TEa for WBC count and platelets was based on recently published data.[30,40]

Table 2. Allowable Total Error (TEa) for Differential Cell Counts. TEobs for differential cell counts should be based on absolute numbers and may be generated using automated techniques, manual techniques, or a combination thereof (see Section 4.2.2). See Appendix A for derivation of TEa values and example calculations.

Measurand	Low value	Within RI	High value	CLIA Value
Neutrophils	15%	15%	15%	+/-3SD
Lymphocytes	15%	15%	15%	+/-3SD
Monocytes	NCR	*60%	50%	+/-3SD
Eosinophils	*90%	50%	50%	+/-3SD
Basophils	Rec Not Possible	Rec Not Possible	Rec Not Possible	+/-3SD

CLIA = Clinical Laboratory Improvement Amendments; NCR = not clinically relevant; Rec = recommendation; RI = reference interval

*Recommendation based on study by Cook, et al.[25]

Table 3 Comparison of Selected Biological Variation Data and ASVCP TEa Recommendations

Biological Variation-Based Quality Specifications Calculated from
Laboratory Beagle Data[a] in Jensen, 1998[45]

| | CVi | CVg | Imprecision (CV %) | | | Bias (%) | | | Total Error (%) | | | ASVCP TEa |
			Opt	Des	Min	Opt	Des	Min	Opt	Des	Min	%
RBC	5.4	4.4	1.4	2.7	4.1	0.9	1.7	2.6	3.1	6.2	9.3	10
HGB	5.9	4.7	1.5	3.0	4.4	0.9	1.9	2.8	3.4	6.8	10.1	10
HCT/PCV	6.4	5.2	1.6	3.2	4.8	1.0	2.1	3.1	3.7	7.3	11.0	10
WBC	12.1	12.3	3.0	6.1	9.1	2.2	4.3	6.5	7.1	14.3	21.4	15[b] 20[c]

Biological Variation-Based Quality Specifications Calculated from
Laboratory Beagle Data (Advia 2120) in Bourgès-Abella, 2015[43]

| | CVi | CVg | Imprecision (CV %) | | | Bias (%) | | | Total Error (%) | | | ASVCP TEa |
			Opt	Des	Min	Opt	Des	Min	Opt	Des	Min	%
RBC	6.0	4.8	1.5	3.0	4.5	1.0	1.9	2.9	3.4	6.9	10.3	10
HGB	6.1	4.4	1.5	3.1	4.6	0.9	1.9	2.8	3.5	6.9	10.4	10
HCT/PCV	6.2	4.3	1.6	3.1	4.7	0.9	1.9	2.8	3.5	7.0	10.5	10
RDW	4.0	6.0	1.0	2.0	3.0	0.9	1.8	2.7	2.6	5.1	7.7	
MCV	2.1	2.6	0.5	1.1	1.6	0.4	0.8	1.3	1.3	2.6	3.9	7
MCHC	2.6	1.1	0.7	1.3	2.0	0.4	0.7	1.1	1.4	2.9	4.3	10
PLT	14.0	15.2	3.5	7.0	10.5	2.6	5.2	7.7	8.4	16.7	25.1	20
WBC	19.6	11.8	4.9	9.8	14.7	2.9	5.7	8.6	10.9	21.9	32.8	15[b] 20[c]
Neut[d]	25.9	14.0	6.5	13.0	19.4	3.7	7.4	11.0	14.4	28.7	43.1	15

ASVCP = American Society for Veterinary Clinical Pathology; CVg = interindividual biological variation; CVi = intraindividual biological variation; Des = desirable; HCT = hematocrit; HGB = hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; Min = minimum; Neut = neutrophils; Opt = optimum; PCV = packed cell volume (spun HCT); PLT = platelets; RBC = red blood cells; RDW = red blood cell distribution width; TEa = allowable total error; WBC = white blood cells;

[a]RBC, WBC, and HGB were determined using a model S560 Coulter Counter; HCT was additionally measured manually using a Haemofuge (a microhematocrit centrifuge). BV-based quality specifications given in these tables were calculated using published biological variation data (as cited) and formulae for optimum, desirable, and minimum thresholds from Fraser, 2001.[15]
[b]Reference laboratories
[c]In-clinic laboratories using point-of-care instrumentation.
[d]Applies to automated absolute neutrophil concentrations.

13 Appendices

13.1 Appendix A. Derivation of hematology TEa recommendations

13.1.1 Selection of measurands

TEa recommendations were made for those hematology measurands in common clinical use and likely to be followed as part of routine quality control procedures. Recommendations were largely based on clinician input for dogs, cats, and horses, and using data generated from QCM and dog specimens. The committee did not make recommendations for mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), or platelet distribution width (PDW), as these were not considered important for this purpose. Recommendations for WBC differential counting (excepting basophils) were made considering automated absolute counts, not differential percentages, since interpretation of patient data and decision thresholds are based on absolute counts. Basophil enumeration was omitted from consideration altogether, as scientific study has documented that commonly used reference laboratory instruments do not enumerate canine and feline basophils accurately.[46] Additionally, the recommendations for the WBC differential were derived from instrument data. Many laboratories standardly perform manual differential cell counts for all patients, and manual differentials should be performed when a review of the smear does not align with the automated differential or if WBC abnormalities are present (e.g. toxic change). However, it is recognized that manual differentials are associated with high imprecision based on the standard 100 to 200-cell differentials commonly performed in veterinary laboratories.[47] Recommendations for reticulocytes were also made considering the absolute count (not percentage), and reticulocytes were only evaluated for purposes of this document using either assayed QCM or canine patient specimens. Because of the difficulty in accurately distinguishing punctate from aggregate reticulocytes in cats using automated methods, specimens from cats were not used.

13.1.2 Clinician input

A total of seven board-certified internists were surveyed (see subcommittee advisors) to determine their expectations of analytical quality required for confident management of their patients (dogs, cats, and horses) using standard diagnostic paradigms. Opinions from all clinicians were surveyed for all measurands. Clinicians were asked to provide the maximum uncertainty they could tolerate clinically for each measurand at low and high concentrations. This assessment was based on values they would deem concerning vs. critical considering the reference intervals for their laboratory. Our goal was to reach a clinical consensus for tolerable analytical error that would result in the fewest mistakes in clinical interpretation. In addition, published hematologic quality requirements were considered from 41 veterinarians representing a

variety of specialties based on maximal allowable deviation from a given result that would not affect their clinical decision.[25] In our experience, the range of clinician expectations was initially quite broad and included small errors that were often unattainable with current instrumentation. However, a consensus was eventually reached that was ultimately more aligned with instrument performance. Cook, et al, observed a much wider decision threshold range, possibly because input was provided without discussion between clinicians and clinical pathologists.[25]

As an example of the discussions, consider desired total error for neutrophil enumeration (Example 1, below). Clinicians, particularly oncologists, often base treatment decisions on a low neutrophil concentration of 2000 cells/µL. Clinicians were asked how much error they could tolerate in making clinical decisions for a patient having a "true" concentration of 2000 neutrophils/µL, and a maximum acceptable range of error around this true value was identified as 1800 to 2200 /µL, or ±200 /µL. Expressed as a percentage, this degree of error is ±10%. While this small degree of error is not attainable with current instrumentation, it provides a goal for manufacturers.

Example 1. Calculation of desired TEa for a moderately decreased absolute neutrophil count

Many reference intervals for neutrophils have a lower limit around 3,000 neutrophils/µL. However, as stated above, clinicians often base treatment recommendations and further diagnostic investigation on the decision threshold of <2,000 neutrophils/µL, with an acceptable range of values at this concentration being 1,800-2,200 neutrophils/µL. Therefore, determination of the desired TEa was based on a decision threshold of 2,000 neutrophils/µL using two different calculations as follows:

TEa based on the lower limit of the reference interval
=[(lower limit of reference interval – decision threshold) / decision threshold] x 100
=[(3,000-2,000)/2,000] x 100
=50%

TEa based on acceptable uncertainty around the decision limit
=[(lower limit of acceptable range – decision threshold) / decision threshold] x 100
=[(1,800-2,000)/2,000] x 100
=10%

This example demonstrates different clinical recommendations for acceptable error in neutrophil count based on either use of the lower limit of the reference interval or the lower limit of the acceptable range around the decision threshold. Widely disparate desired TEa were obtained, but given the importance of the clinical decisions being made at such low neutrophil counts, we considered 10% as the desired TEa, which is based on

clinically important values unrelated to a reference interval. This clinician input was considered in combination with instrument performance to determine the recommended TEa (see below).

Example 2. Calculation of desired TEa for a moderately decreased platelet count

Many reference intervals for canine platelet counts have a lower limit around 200,000/µL. However, many surgeons have a minimal threshold of 100,000/µL for performing a major surgical procedure. Clinicians identified an acceptable range of values at this concentration to be +/- 15,000 platelets (85,000-115,000 platelets/µL). Therefore, determination of the desired TEa was based on a decision threshold of 100,000 platelets/µL, as follows:

TEa based on acceptable uncertainty around the decision limit
=[(lower limit of acceptable range − decision threshold) / decision threshold] x 100
=[(85,000 - 100,000) / 100,000] x 100
=15%

Therefore, the desired TEa for a moderately low platelet concentration was calculated as 15%.

13.1.3 TEa based on assessment of instrument performance

Performance of reference laboratory hematology instruments was performed using College of American Pathologists (CAP) EQA/PT data as well as historical QC data from each institution. Four institutions participated in the same CAP proficiency testing event (September 2012) and additionally measured selected CAP test material (one or more materials chosen from the 5 different test specimens provided for the testing event) 5 times under repeatability conditions. Repeatability data were used to calculate CV; difference from peer group mean was used to calculate %bias. TEobs was calculated according to the formula TEobs = %bias + 2CV. TEobs values varied by measurand concentration; in general, higher TEobs values were observed at lower measurand concentrations.

Additionally, historical daily measurements of QCM over 15-40 days were used to calculate CV, %bias, and TEobs at the institutions above plus additional institutions recruited by the committee. Percent bias was calculated using expected means reported by the QCM manufacturer. TEobs was calculated as above. TEobs values varied by measurand concentration; in general, higher TEobs values were observed at lower measurand concentrations. The range of TEobs based on both CAP proficiency testing and historical control data are summarized in Table A1a below.

13.1.4 Determination of recommended TEa.

TEa recommendations were based on collective consideration of clinician desired total error, instrument performance, and CLIA guidelines. Final recommendations are the consensus opinion of committee members based on the above factors. While the analytical performance of many of the measurands was well within clinicians' expectations for quality requirement, some could not be achieved based on analytical performance of current, commonly used instruments, particularly at extremes of values. For instance, in the clinician's desired TEa calculation for neutrophils presented above, the desirable TEa was 10%. However, reference laboratory instrumentation could not reliably meet this desired TEa, and our recommended TEa is therefore ≤15%. Both decision thresholds and instrument performance are expected to evolve over time, as disease diagnosis is refined and technology evolves. Consequently, these recommendations will be reviewed and revised every ten years.

Table A1a. Derivation of TEa recommendations including comparison of total allowable error (TEa) with total observed error (TEobs), clinician error limit, and Clinical Laboratory Improvement Amendments (CLIA) for hematology measurands.

Measurand	TEa	TEobs[a]	Clinician Error Limit[b]	CLIA Value
RBC	10%	1-8%	10-20%	6%
Hgb	10%	2-7%	10-15%	7%
Hct/PCV	10%	1-8%	5-10%	6%
MCV	7%	1-3%	5-15%	---
MCHC	10%	2-3%	5-15%	---
Reticulocytes	20%	10-56%[c]	10-15%	---
Platelets	20%[d] 25%[e]	5-15%[d] 6-41%[e]	15-20%	25%
WBC	15%[d] 20%[e]	1-13%[d] 6-16%[e]	10-15%	15%
Neutrophils	15%	7-11%	10-15%	+/-3SD
Lymphocytes	15%	8-14%	10-15%	+/-3SD
Monocytes	60% (within RI) 50% (above RI)	14-37%	3-200%[25]	+/-3SD
Eosinophils	90% (below RI) 50% (within/above RI)	16-38%	5-200% [25]	+/-3SD

CLIA = Clinical Laboratory Improvement Amendments; HCT = hematocrit; HGB = hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; PCV = packed cell volume (spun HCT); RBC = red blood cells; TEa = total allowable error; TEobs = total observed error; WBC = white blood cells

[a]TEobs is based on reference laboratory data (7 different instruments from 3 different manufacturers). The range provided is a summary of all control levels used, including both historical QCM controls as well as prospective CAP QCM testing, as described in the text.
[b]Clinician error limit refers to the acceptable error that clinicians are willing to tolerate in a measurement that will not impact diagnostic or therapeutic decision making.
[c]The large TEobs for reticulocytes was observed for QCM that had low numbers of reticulocytes (See Table A1b).
[d]Reference laboratory instruments
[e]In-clinic instruments. Data based on two in-clinic instruments.

Table A1b. Levels/concentrations of historical QCM used by the institutions that provided data for this study and the interval of TEobs for each using reference laboratory instrumentation.

	Low		Mid		High	
	Value	**TEobs**	**Value**	**TEobs**	**Value**	**TEobs**
RBC (x 10^9/µL)	2.3-2.9	2-6%	4.2-4.5	1-5%	5.2-5.4	1-8%
Hgb (g/dL)	5.4-7.5	2-7%	11.6-12.3	1-4%	16.1-17.1	1-6%
Hct/PCV (%)	16-23	2-6%	33-37	2-5%	46-49	1-8%
MCV (fL)	70-79	2-3%	78-88	1-3%	89-94	1-3%
MCHC (g/dL)	32.6-32.8	2-5%	32.7-34.1	2%	33.2-34.9	2-3%
Reticulocyte %	0.7-1.4	17-56%	1.8-4.6	8-16%	5-8.7	6-18%
*Reticulocyte (x 10^3/µL)	30-50	17-56%	80-200	12-17%	110-220	10-19%
Platelets (x 10^3/µL)	60-80	7-15%	215-230	4-10%	450-550	5-14%
WBC (x 10^3/µL)	3-4	3-13%	7.1-7.2	1-10%	16-18	3-12%

HCT = hematocrit; HGB = hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; PCV = packed cell volume (spun HCT); RBC = red blood cells; TEobs = total observed error; WBC = white blood cells

*Note that a recommendation for TEa for low reticulocyte concentrations was not deemed clinically relevant.

13.2 Appendix B. External quality assurance/proficiency testing programs

Vendors and manufacturers are listed alphabetically. This list is for informational purposes only and does not constitute a legal contract or endorsement between ASVCP and any person or entity unless otherwise specified. The ASVCP does not endorse any particular vendor or manufacturer.

CLIA
https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Proficiency_Testing_Providers.html

College of American Pathologists
http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation

Insight Interlaboratory Quality Assessment Program from Sysmex
https://www.sysmex.com/us/en/Pages/Registration.aspx

Urika, LLC Quality Assurance Division
www.urikapathology.com

Veterinary Laboratory Association Quality Assurance Program
http://www.vlaqap.org/

13.3 Appendix C. Allowable total error worksheet

1. Calculate analyzer precision by performing a minimum of n=5 repetitions. (More repetitions are preferred.) Repetitions should be performed identically to standard analysis of patient specimens, using the same personnel, instrument, reagents, etc. Calculate mean, standard deviation, and coefficient of variation (CV%) using any standard software program (e.g., Excel):

$$CV\ (\%) = \frac{Standard\ deviation}{Mean} \times 100$$

2. Calculate analyzer bias from known standard/control material ($Mean_{target}$) or deviation from population mean and measured mean from Step #1 ($Mean_{measured}$) using equation:

$$Bias(\%) = \frac{Mean_{target} - Mean_{measured}}{Mean_{target}} \times 100$$

3. Calculate Total Error (TE_{obs}) using the following formula. Use the absolute bias result (i.e., positive number only).
$$TE_{obs} = absolute\ bias\% + 2CV$$

4. Compare TE_{obs} calculated in the laboratory with TE_a found in the ASVCP Guidelines for TEa.
    a. If $TE_{obs} \le TE_a$
        i.   The quality assessment passes and no further action is needed
    b. If $TE_{obs} > TE_a$
        i.   Report results to Quality Assurance Personnel/Committee.
        ii. Investigate pre-analytical and procedural factors (e.g., specimen quality, instrument SOP, operator proficiency, bias determination) that may have impacted performance and correct as needed. Reassess instrument performance following correction.
        iii. If no pre-analytical or procedural factors are identified that can be addressed and corrected, management should report findings to the manufacturer of the instrument so that any needed maintenance, repairs, or replacement may be evaluated and implemented.
        iv. Clinicians should be notified in writing of potentially clinically impactful error.

5. All Total Error assessments should be catalogued in a written and/or digital archive accessible to personnel who may operate the instrument or interpret the results.[13]