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SPECIAL REPORT

ASVCP guidelines: allowable total error guidelines for biochemistry

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Abstract: As all laboratory equipment ages and contains components that may degrade with time, initial and periodically scheduled performance assessment is required to verify accurate and precise results over the life of the instrument. As veterinary patients may present to general practitioners and then to referral hospitals (both of which may each perform in-clinic laboratory analyses using different instruments), and given that general practitioners may send samples to reference laboratories, there is a need for comparability of results across instruments and methods. Allowable total error (TE_a) is a simple comparative quality concept used to define acceptable analytical performance. These guidelines are recommendations for determination and interpretation of TE_a for commonly measured biochemical analytes in cats, dogs, and horses for equipment commonly used in veterinary diagnostic medicine. TE_a values recommended herein are aimed at all veterinary settings, both private in-clinic laboratories using point-of-care analyzers and larger reference laboratories using more complex equipment. They represent the largest TEa possible without generating laboratory variation that would impact clinical decision making. TE_a can be used for (1) assessment of an individual instrument's analytical performance, which is of benefit if one uses this information during instrument selection or assessment of in-clinic instrument performance, (2) Quality Control validation, and (3) as a measure of agreement or comparability of results from different laboratories (eg, between the in-clinic analyzer and the reference laboratory). These guidelines define a straightforward approach to assessment of instrument analytical performance.

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Position Statements and Special Reports developed by the American Society for Veterinary Clinical Pathology (ASVCP) provide current information on topics in veterinary clinical pathology that are important to the veterinary community. The procedure for submitting statements is detailed at www.asvcp.org/membersonly/positionpapers.cfm. The ASVCP Executive Board is responsible for the review and approval of all statements, often following a period of input from the ASVCP membership and experts in the field. The final draft is then submitted to *Veterinary Clinical Pathology* and is edited prior to publication.

Introduction

Quality assurance (QA) includes selection and evaluation of instrument/method performance in order to ensure that validated, reliable, and robust methods appropriate for the species being tested are in use.[1] In-clinic quality control (QC) validation and reference interval generation are part of the instrument/method validation and verification process.[2] Once instruments/methods/operator are judged to be suitable for routine testing, QA ensures that ongoing instrument performance is stable and that the errors inherent to the instrument/methods do not exceed levels that would invalidate the interpretation of test results. Important QA tools include regular QC procedures and participation in an external QA/proficiency testing (EQA/PT) program. For a glossary listing used terms and definitions please consult Appendix 1.

The concept of quality requirements is the foundation for quality planning. Quality requirements can help guide interpretation of laboratory test results because they provide a perspective about variability of results within an acceptable interval and potential significance of abnormal findings. A hierarchy of quality requirements has been proposed[3], and the most stringent quality requirements are based on clinical outcomes and clinical decision thresholds. Quality requirements may also be based on data about biologic variation of a measurand, analytical performance criteria (eg, as mandated by Clinical Laboratory Improvement Amendment [CLIA] for human medicine), expert opinion and/or technological state-of-the-art.[3]

A commonly used quality requirement is allowable or desirable total error (TE_a), which is derived from medically important measurand or clinical decision thresholds. Westgard was the first to introduce the concept of total error (TE) in 1974.[4,5] Analytical imprecision (reproducibility of the result) and bias (systematic error) were combined into a single measure of the uncertainty of a test result. The ideal situation is to have highly accurate and precise measurement, ie, low bias and low coefficient of variation (CV) or standard deviation (SD), respectively. Westgard originally used TE = bias(%) + 1.65CV, but a coefficient for the CV as high as 6 has been used by some authors for method validation studies.[6] In order to better define TE_a for the veterinary community with consideration of diagnostic needs and reference intervals of common species, the Quality and Laboratory Standards (QALS) Committee of the American Society for Veterinary Clinical Pathology (ASVCP) formed a methods validation subcommittee in 2009 to develop guidelines for total error in veterinary medicine. For the purpose of this document, we define TE_a as bias (%) + 2CV(%) which is consistent with CLIA recommendations.[7] If units of the test are used, then the equation, bias (expressed in units of the test) + 2 Standard Deviations (SD), is used to calculate absolute TEa.

TE_a can be used to aid instrument selection if manufacturer's claims for instrument performance are available. TE_a can also be compared to an instrument's calculated or observed total error (TE_{obs}) to help determine whether that instrument's analytical performance is adequate.[8] If analytical performance is deemed adequate, TE_a can further be used during QC validation of that instrument. Use of TE_a for internal QC validation is not explained fully in this document, but further detail is available in the ASVCP QALS Quality Assurance for Point-of-Care Testing in Veterinary Medicine.[9] Finally, TE_a can be used to guide comparison of test results across laboratories and clinics using the same or different analytical methods. TE_a can be used to help interpret results from external QA (proficiency testing) programs or to help interpret results of comparability testing, where a reference laboratory is used to check in-clinic or other laboratory results. Additional detail on recommended TE_a use can be found in the ASVCP QALS General Quality Assurance Guidelines and ASVCP QALS Quality Assurance for Point-of-Care Testing in Veterinary Medicine.[1,9]

It is important to realize that TE_a may differ with species, analyte concentration, clinical use, or type of laboratory. TE_a for a given analyte may be different for dogs vs cats vs horses, dependent on different diseases found and therapies applied in a species. TE_a may differ at low, within reference interval, or high analyte concentrations based both on clinical decision making and realistic analytic performance. TE_a may differ at different cut-off values for "normal" or at different clinical decision thresholds. TE_a for the same analyte may differ for reference laboratories, veterinary practices, toxicology laboratories, and other industry laboratories, as these serve different animal populations, different clinical and research needs, and may use different instruments and methods.

Quality Control Material

The purpose of analyzing quality control material (QCM) is to detect excessive analytical errors that deviate from expected instrument performance, given stable operation in a routine setting.[10] Comparability testing among methods or instruments is used to investigate external quality assessment failure (eg, disparate results between the clinic or laboratory's mean and

the peer group mean). Common causes of non-comparability of results (results for the same sample that differ significantly) include sample transport and storage effects as well as other pre-analytical parameters, instrument failure or drift, reagent degradation, differences in calibration, imprecision, different analytical methodologies, and others.

Both in-clinic QC and EQA/PT require different levels (concentrations, activities, etc) of measurement dependent upon the instrument, reference intervals, expected changes due to disease, and species evaluated at the facility. All commercially available QCM have a lot number (batch number) and expiration date. QCM degrade over time, and expiration dates must be strictly observed. QCM should be stored and handled appropriately, as directed by the manufacturer.[11] QCM from different lots may not have the exact same analyte concentrations. This impacts QC because it alters the control limits used to decide if QC data are in or out of control.[10]

In a previous study, a minimum of 2 levels (analyte concentrations/activities) of assayed QCM were found to be adequate for external quality assessment in the form of instrument/method performance valuations for in-clinic analyzers.[12] Evaluation of these materials with levels of analytes at or near those of clinical decision values and/or reference interval limits should be conducted at least biannually. If a sufficient supply of QCM can be reserved for an entire year, use of the same QCM for the entire year is ideally recommended.

Artificially prepared QCM may not behave in exactly the same manner as patient samples. However, as QCM are likely to be more stable than patient samples, these may be preferred to assess reagent or instrument drift. Multiple factors may influence the choice of the assayed QCM, including but not limited to, commutability across instruments and methods, numbers, types and levels of analytes present within the materials, shelf-life or stability, cost, and other factors as determined by the laboratory director.

Handling and Transport

The stability of external QCM during transport to the laboratory and storage within the laboratory need to be carefully considered. QCM that are transported or stored under inappropriate conditions may lead to errors during comparability studies. Conditions of storage, including temperature, light, humidity, duration, etc, must be monitored to ensure stability of QCM, and conditions known to alter the stability of the QCM should be avoided. For example, repeat freeze-thaw cycles encountered with frost-free freezers lead to degradation of QCM analytes and should be avoided. Instead, liquid QCM typically are stored in −70°C freezers to ensure stability for extended time of storage. Manufacturer's recommendations regarding storage should be followed and included in Standard Operating Procedures (SOPs). All QCM should be labeled with an expiry date and promptly discarded upon reaching this date. No expired QCM or reagents (including rotors or cartridges) should be used. If the stability of the QCM is suspected to be compromised, it should be discarded and replaced. Backdating of instruments to use outdated reagent or QCM should never be performed. Even under appropriate conditions of transport and storage, analyte results may vary over the lifetime of the QCM while yet remaining within the expected intervals. Therefore, when performing comparability studies on different analyzers, QCM should be analyzed at approximately the same time. This may require splitting and shipping of QCM in aliquots instead of analyzing the QCM on different days at different facilities.

Transportation of products with variable environmental conditions and time to measurement also may change concentrations of the analytes. These must be controlled and, if there is known compromise of the QCM, eg, excessive heating, repeated freeze-thaw cycles, etc, the QCM should be replaced. Additionally, if different analyzers are to be compared, it is recommended to prepare aliquots and coordinate the testing so all aliquots are measured at approximately the same time, ie, within 4–6 hours. In the authors' experience with in-clinic QA at veterinary clinics, procurement of frozen liquid control specimens is preferable rather than lyophilized control materials that need to be reconstituted. This helps minimize pipetting, contamination, or other source of errors that may be introduced by dilution of lyophilized control materials.

Frequency

In order to ensure ongoing production of reliable laboratory results that enable quality diagnostic medicine over time on the same or between different instruments, both in-clinic or within laboratory QC and EQA/PT must be performed regularly in order to detect changes in analyte measurement resulting from pre-analytical, analytical, or post-analytical error.

Recommended frequency of monitoring may vary between laboratories and clinics. In general, frequent internal QC (daily or weekly) and periodic EQA/PT (quarterly) should be performed.[13] Special cause testing may be initiated if there is suspicion of inaccurate measurement of patient samples and concern for accurate diagnosis. While special cause testing and a subsequent quality investigation may successfully identify sources of error, laboratories and clinics are encouraged not to rely on special cause testing as their sole method of quality assessment. Special cause testing is not adequate as the sole method of quality assessment for any instrument as there is significant likelihood of misdiagnosis of patients.

Periodic QC is performed when frequent monitoring is deemed unnecessary because the measurement systems involved are stable and the risk of errors in the clinical interpretation due to noncomparable (disparate) results is low. Determination of stability should be assessed with knowledge of the assay, knowledge of reagent stability, clinical experience and consistent monitoring of the assay for a minimum of 30 days. Special cause testing is performed in response to an alert from a monitoring procedure or another triggering event.[14] The frequency of external QA monitoring/proficiency testing 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. Reference laboratories are likely to have a QA specialist on staff who can make decisions about frequency of proficiency testing; private practices are encouraged to consult with the instrument manufacturer or a board-certified veterinary clinical pathologist with expertise in QA.

Instrument Performance Evaluation: Calculation of CV, SD, Bias, and Observed Total Error

More 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 QC specialist. The following guidelines are provided for in-clinic laboratories, but many of the general principles apply to all types of laboratories conducting veterinary blood sample testing.

Instrument performance evaluation may be performed when a new instrument is being considered for purchase, a new instrument is evaluated to ensure that it performs according to manufacturer's claims during warranty, there is routine internal QC of an instrument conducted to ensure adequate ongoing performance, or when evaluating performance as part of an EQA/PT program.

CV for the purpose of calculation of the observed total error (TE_{obs}) should be determined from precision studies using QCM at various levels of analytes, known standards, or assayed patient samples. Three methods that may be used to determine CV and bias include:

1. Comparison with target values provided by manufacturers of assayed QCM. An assayed QCM may be repeatedly measured for 5 days to determine mean, bias, SD and CV. In this situation, the mean of the results should be compared to the assayed mean to determine bias. These data can then be used to calculate TE_{obs} of the analyte. The assayed QCM should be specific for the equipment and the methods being evaluated; the instrument manufacturer should be consulted if there is any doubt regarding QCM suitability. Please consult the manufacturer to insure that it is appropriate for the equipment and the methods.
2. Comparison with known gold standards for various analytes (standards provided by external regulating or governmental agencies or other specialist resources)
3. Comparison with peer group means in an EQA/PT program. This typically must be done using an EQA program that is employed to help insure quality laboratory results. While some EQA programs in human medicine use assayed QCM, veterinary EQA programs typically use unassayed QCM and rely on the peer group mean to determine laboratory performance. A peer group is defined by use of the same instrument and method as that upon which the result is obtained. EQA using comparison with a peer group is dependent on inclusion of sufficient numbers of instruments included in the peer group as well as other laboratories' maintenance of equipment and QC. This approach is best suited for ongoing instrument performance evaluation following initial and annual instrument evaluations conducted using assayed QCM which has a known target mean or known standards.

As EQA programs currently in existence are method specific, and methods used by in-clinic laboratories are frequently not represented at the time of publication of this document, option 3 is often not available to the in-clinic veterinary laboratory.

Instrument Performance Evaluation Steps

The following steps are designed for QA assessment for the in-clinic laboratory, but may also be used as a very basic guideline by reference laboratories. Further information for complete review by reference laboratories is available at www.westgard.com, in the ASVCP General QA guidelines,[1,2,15,16] and in Clinical Laboratory Standards Institute documents, as needed. All steps should be carried out by appropriately trained personnel who are knowledgeable regarding the analyzer's operation and the facility's QA program. Calculations can easily be carried out using commercially available software programs. Calculations should be performed for each analyte and each QCM as follows:

1. Measure each QCM daily for a minimum of 5 days.[10] Five repetitions in one day is a possible alternative but does not incorporate potential interday variation that may be found when assessing samples from hospitalized patients. Using these data, for each QCM and each analyte, calculate
   a. Mean (average)
   b. SD
   c. CV, representing between-day (interassay) imprecision of the analyzer.

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

   The SD and CV of the analyzer derived from these QC data are referred to as the calculated or observed SD, and CV.
2. Calculate the analyzer's measured bias using the measured mean and the QCM manufacturer's reported mean for the assayed control material (using the same instrument and/or method as that used by the analyzer), according to the formula:

   $$\mathrm{Bias}\% = \frac{\mathrm{Mean}_{\mathrm{manufacturer}} - \mathrm{Mean}_{\mathrm{measured}}}{\mathrm{Mean}_{\mathrm{manufacturer}}} \times 100$$

   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 (eg,−5.0%), then the *absolute number* (5.0%) should be used in step 4, below.
3. Calculate the analyzer's TE_{obs} using measured CV and measured bias, according to the formula:

   $$\mathrm{TE}_{(\mathrm{obs})} = 2\mathrm{CV} + \mathrm{Bias}\%$$

4. Compare measured TE_{obs} to TE_a. If $TE_{obs} < TE_a$ (or very close to it), then the quality requirement is met and the instrument and method are considered suitable for measurement of that analyte. If $TE_{obs} > TE_a$, then several options exist (see below).

TE_{obs} Interpretation and Assessment of EQA Results

TE_{obs} for all analytes determined on in-house or reference lab equipment should be compared to the TE_a proposed by the ASVCP subcommittee listed in Table 1. If TE_{obs} for all concentrations is less than that which is allowable (TE_a), then instrument performance is satisfactory and no further assessment for that analyte is required. If TE_{obs} is greater than TE_a, attempts should be made to identify and correct the potential causes of imprecision (high CV) and inaccuracy (high bias).[13] Use of special calculations, such as the Quality Goal Index[17,18] may be helpful in determining if the poor performance is due to imprecision, bias, or a combination thereof.

If the sources of observed 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 by analyzer adjustments, operator training, replacement of a particular reagent with new reagent or a product from a different manufacturer, or, potentially, analyzer replacement. Alternately, the initial quality requirements may be relaxed. This is not recommended but is possible only if a potential additional error can be tolerated in diagnostic judgment. This requires education of all clinicians using the analyzer regarding amended TE_a of the analyte(s) in question. Any changes outside of the recommended TE_a in this document must be justified and documented in a laboratory handbook. This should be done only upon consultation with a board-certified veterinary clinical pathologist.

Total Allowable Error Specifications Based on Clinical Decision Limits

In order to establish the TE_a guidelines provided in this document, clinicians were surveyed to determine their expectations of analytical quality required for confident management of their patients using standard diagnostic paradigms (see Acknowledgments in Data S1

Table 1. Allowable total error (TE_a) for biochemical analytes.

Analyte	Low Analyte Values	Within RI	High Values	CLIA Value
Albumin	15%	15%	15%	10%
ALP	NCR	25% (20% desirable)	25% (20% desirable)	30%
ALT	NCR	25%	25%	20%
Ammonia	NCR	20%	20%	Not found
Amylase	NCR	25%	25%	30%
AST	NCR	30%	30%	20%
Bicarbonate	20% (15% desirable)	20% (15% desirable)	20% (15% desirable)	10% (RCPA) to 20% (CAP)
Bile Acids[19–21]	20%	20%	20%	None found
Cholesterol	20%	20%	20%	10%
Chloride	5%	5%	5%	5%
CK	NCR	30%	30%	30%
Creatinine[22]	20%	20%	20%	15%
GGT	NCR	20%	20%	15% (RCPA) to 30% (CFX)
GDH	NCR	30%	25%, >90 IU 20%	None found
Glucose	10%	20%	20%	6% Low, 10% High
Iron	30% (15% desired)	30%	30%	20%
Potassium[23]	10%	5%	5%	0.5 mmol/L
Lactate[23–25]	NCR	40%	40%	10 (RCPA) to 30% (CFX)
LDH	NCR	20%	20%	20%
Magnesium[26–28]	15% desirable, 20% acceptable	15% desirable, 20% acceptable	15% desirable, 20% acceptable	25%
Sodium[23]	5%	5%	5%	4 mmol/L
Phosphorus[29]	20%	15%	15%	10–23% (CAP)
SDH	NCR	25%	25%	None found
Total Bilirubin	NCR	30% (25% desirable)	30% (25% desirable)	0.4 mg/dL, 20%
Total Ca[27–29]	10%	10%	10%	2% (BV) to 8% (CFX)
Total Protein	10%	10%	10%	10%
Triglyceride	NCR	25%	25%	25%
Troponin[30]	NCR	70%	70%	20%CV maximal with around 50% TEa if calculated
Urea[31]	15%	12%	12%	2 mg/dL, 9%
Uric acid[32]	10%	10%	10%	17%

NCR indicates not clinically relevant. Three to 5 board-certified clinicians (ACVIM or ECVIM with various specialties) gave opinions upon clinically desired TE_a for low, mid, and high analyte concentrations and activities, except for troponin I where the opinion of a single cardiologist was considered (see Acknowledgments in Data S1). TE_{obs} was calculated directly from reference equipment used by QALS members using the equation $2CV + bias\% = TE_a\%$ to ensure that calculation of TE_a was possible. CAP, College of American Pathologists Participant Summary, April 2004. CLIA, Clinical Laboratory Improvement Amendment '88 Proficiency Testing Limits, U.S. Federal Register. BV, Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC) Table of Desirable Quality Specifications based on Biological Variation, 2004 Update. For details, visit www.westgard.com/guest26.htm (accessed October 2013) and http://www.dgrhoads.com/db2004/ae2004.php (accessed October 2013). CFX – Canadian Fixed Limits, The College of Physicians and Surgeons of Saskatchewan. RCPA, Royal College of Pathologists of Australasia and the Australasian Clinical Biochemist association Quality Assurance Program.

online resource). The goal was to reach a clinical consensus that would result in minimal alpha or beta error as a result of analytical error. The advantage of this approach was that it was based on clinical experience and therefore acceptable to clinicians in their diagnostic framework. However, some clinicians' expectations for quality requirement could not be achieved based on analytical performance of current, commonly used instruments. Therefore, some of the TE_a listed in Table 1 (eg, ALP and magnesium) reflect the current technological state-of-the-art rather than clinicians' requirements. Additionally, some clinical decision limits are anticipated to change over time, as analytical performance improves and disease diagnosis is further refined. Consequently, these recommendations will be reviewed and revised in the future.

Bias, CV, and TE_a Specifications Based on Biologic Variation

Biologic variation may also be used to establish bias, CV, and TE_a.[19] While biologic variation was considered in selection of quality requirements, for several

Table 2. Recommendations for analytical coefficient of variation, bias and total error based on biologic variation of various biochemical and haematological analytes in dogs.

Information from the Literature[A–N]				Traditional Quality Specifications Based on Biologic Variation[21,22,23]									Alternative TE Based on Biologic Variation[19]			
Analyte	CVg = Betwdog[20]	CVi = Withindog[20]	CVa[20]	CV Opt[A]	CV Des[B]	CV Min[C]	Bias Opt[D]	Bias Des[E]	Bias Min[F]	TE Opt[G]	TE Des[H]	TE Min[I]	Alt TE Opt[J]	Alt TE Des[K]	Alt TE Min[L]	Index of Individuality[M] (Category[N])
RBC	4.4	5.4	2.8	1.35	2.70	4.05	0.87	1.74	2.61	3.10	6.20	9.29	2.23	4.46	6.68	0.73 (Intermediate)
Hct	5.2	6.4	1.1	1.6	3.20	4.80	1.03	2.06	3.09	3.67	7.34	11.01	2.64	5.28	7.92	0.80 (Intermediate)
Hgb	4.7	5.9	2.9	1.48	2.95	4.43	0.94	1.89	2.83	3.38	6.76	10.14	2.44	4.87	7.31	0.72 (Intermediate)
WBC	12.3	12.1	3.7	3.03	6.05	9.08	2.16	4.31	6.47	7.16	14.29	21.45	5.00	9.98	14.98	0.97 (Intermediate)
ALT	23.7	9.7	3.2	2.43	4.85	7.28	3.20	6.40	9.60	7.21	14.40	21.61	4.01	8.00	12.01	2.32 (High)
AST	10.9	11.4	3.3	2.85	5.70	8.55	1.97	3.94	5.91	6.67	13.35	20.02	4.70	9.41	14.11	0.92 (Intermediate)
ALP	34.2	8.6	1.7	2.15	4.30	6.45	4.41	8.82	13.22	7.96	15.92	23.86	3.55	7.10	10.64	3.90 (High)
Alb	3.0	2.4	1.6	0.60	1.20	1.80	0.46	0.93	1.39	1.45	2.91	4.36	0.99	1.98	2.97	1.04 (Intermediate)
TP	3.1	2.6	1.1	0.65	1.30	1.95	0.51	1.01	1.52	1.58	3.16	4.74	1.07	2.15	3.22	1.10 (Intermediate)
Creatinine	12.9	14.6	2.9	7.30	3.65	10.95	2.44	4.88	7.31	8.46	16.93	25.38	6.02	12.05	18.07	0.87 (Intermediate)
Cholesterol	15.1	7.3	3.0	1.83	3.65	5.48	2.10	4.19	6.29	5.12	10.22	15.33	3.02	6.02	9.04	1.91 (High)
Glucose	3.8	9.5	3.7	2.38	4.75	7.13	1.28	2.56	3.84	5.21	10.40	15.60	3.93	7.84	11.76	0.37 (Low)
Fructosamine	4.2	11.1	2.8	2.78	5.55	8.33	1.48	2.97	4.45	6.07	12.13	18.20	4.59	9.16	13.75	0.37 (low)
Potassium	3.6	3.3	0.1	0.83	1.65	2.48	0.61	1.22	1.83	2.07	3.94	5.92	1.37	2.72	4.09	1.09 (Intermediate)
Total T4	17.2	17.0	4.0	4.25	8.50	12.75	3.02	6.05	9.07	10.03	20.08	30.11	7.01	14.03	21.04	0.99 (Intermediate)
Ctsh	43.6	13.6	8.8	3.40	6.80	10.20	5.71	11.42	17.13	11.32	22.64	33.96	5.61	11.22	16.83	2.69 (High)
Iron	17.2	17.8	0.7	4.45	8.90	13.35	3.09	6.19	9.28	10.43	20.88	31.31	7.34	14.69	22.03	0.97(Intermediate)
Fibrinogen	19.0	17.1	2.8	4.28	8.56	12.84	3.20	6.39	9.59	10.26	20.51	30.78	7.06	14.12	21.19	1.10 (Intermediate)
CRP	29.3	24.3	7.2	6.08	12.16	18.24	4.76	9.52	14.27	14.79	29.58	44.37	10.03	20.06	30.10	1.16 (Intermediate)
a-1-AGP	67.0	9.6	8.1	2.40	4.80	7.20	8.46	16.92	25.38	12.42	24.84	37.26	3.96	7.92	11.88	5.33 (High)
Haptoglobin	20.2	17.0	4.9	4.25	8.50	12.75	3.30	6.60	9.90	10.31	20.63	30.94	7.01	14.03	21.04	1.14 (Intermediate)

CV indicates coefficient of variation; CVa, analytical CV; CVi, within dog CV; CVg, between dog CV.

A = CV Opt = recommended optimal analytical CV based on CVa < 0.25CVi.

B = CV Des = recommended desirable analytical CV based on CVa < 0.5 CVi.

C = CV min = recommended minimally acceptable analytical CV based on CVa < 0.75 CVi.

D = Bias opt = recommended optimal Bias based on $B < 0.125(CVi^2 + CVg^2)^{1/2}$.

E = Bias Des = recommended desirable Bias based on $B < 0.250\ (CVi^2 + CVg^2)^{1/2}$.

F = Bias min = recommended minimally acceptable Bias based on $B < 0.375(CVi^2 + CVg^2)^{1/2}$.

G = TE Opt = recommended optimal TE based on $TEa < 1.65\ (0.25CVi) + 0.125\ (CVi^2 + CVg^2)^{1/2}$.

H = TE Des = recommended desirable TE based on $TEa < 1.65\ (0.50CVi) + 0.250\ (CVi^2 + CVg^2)^{1/2}$.

I = TE min = recommended minimally acceptable TE based on $TEa < 1.65\ (0.75CVi) + 0.375(CVi^2 + CVg^2)^{1/2}$.

J = Alt TE Opt = recommended optimal TE based on TEa < 1.65 (CV Opt).[19]

K = Alt TE Des=recommended desirable TE based on TEa ≤ 1.65 (CV Des)).[19]

L = Alt TE min = recommended desirable TE based on TEa < 1.65 (CV Min)).[19]

M = Index of individuality; (II) = CVb/square root of (CVi2 + CVa2). Interpretation: > 1.7 = high index of individuality; reference change value likely to provide better determination of significant difference in sequential analyses; use of 95% population-based reference interval may hamper diagnostic sensitivity. < 0.7 = low index of individuality; use of 95% population-based reference interval is valid. Between 0.7 – 1.7 = reference change value may be of benefit in determining significant difference in sequential analyses. Note: the II will change with assays having a CVa different from that presented in this table.[35]

N = Categories of II = High (> 1.7), Intermediate (0.7–1.7), Low (< 0.7).

analytes, such as ALT, a biologic variation-based quality requirement was too stringent given the analytical performance that is currently possible with most diagnostic instruments. Therefore, while data on biologic variation are presented in Table 2 for informational purposes, these are not recommended as guidelines to ensure adequate instrument performance. Some instrument or method performance may be achievable using these calculated specifications, while others may not. Improvements in instrument and method performance in the future may allow improved quality requirements based on biologic variation. The data in Table 2 are based on studies conducted in dogs, primarily by a single group of clinical researchers. Additional studies in other species may help us better understand the needs for instrument and method performance of other species.

Summary

TE_a is a simple comparative quality concept used to define acceptable analytical performance. It is defined as Bias% + 2CV for most purposes in veterinary medicine. TE_{obs} of an instrument in any diagnostic or research setting may be compared to the recommended TE_a determined by veterinary specialists found in Table 1 to evaluate manufacturer claims, new and warrantied instruments, in EQA/PT, comparability testing, and potentially other components of a QA plan. If $TE_{obs} < TE_a$, the instrument is functioning within ASVCP recommended guidelines. If $TE_{obs} >$ TEa, the manufacturer and/or a board-certified clinical pathologist with expertise in QA should be consulted to troubleshoot potential problems with fluidics, electronics, lighting, the computer/software, reagents, QCM, or other potential instrument error.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Web Resources and Acknowledgments.

Appendix 1

Glossary of Terms

Accuracy – Closeness of agreement between the results of a measurement and the true concentration of the analyte. Accuracy is the opposite of inaccuracy, or bias.
Alpha Error – Probability of falsely rejecting the null hypothesis (typically defined as health in medicine) when it is true; false positive.
Beta Error – Probability of falsely rejecting the alternative hypothesis (typically defined as disease) when it is true; false negative.
Bias (a.k.a. inaccuracy) – Total systematic error, which includes constant and proportional bias. Bias is the difference between the measured result and some measure of the "true" value (e.g, as measured by a reference method or as defined by a known standard). The term *bias* has a specific meaning in the statistical t-test and in difference plot analysis, where bias (expressed in analyte units) equals the difference between the mean values of 2 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

$$\text{Bias}\% = \frac{\text{Mean}_{\text{target}} - \text{Mean}_{\text{measured}}}{\text{Mean}_{\text{target}}} \times 100$$

Bias can be positive or negative; when used to calculate observed total error, the absolute value is used.

Recommendations made in this guideline focus on using a known mean concentration of commercially available assayed control material as the target mean, since control materials are most easily accessible and cost-effective for privately practicing veterinarians. In clinical pathology laboratories, best practice dictates that target means be based on data from method comparison to a true reference method ("definitive" method) or known concentration of certified reference material.[1,2] Target means may also be based on peer group means from external quality assessment (EQA, or proficiency testing) program data.

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

Bias, proportional – When the magnitude of systematic error changes as the analyte concentration changes. Often, error increases as the analyte concentration increases, but the reverse may also be true.[3]

Calibration – The process of testing and adjusting how a laboratory instrument or test system measures a substance by comparing it to a known substance (the calibrator) and subsequently defining the association between the instrument/test system and the value of the calibrator.

Calibrator – A material intended by its manufacturer to be used to define the association of a laboratory instrument measurement to a known value. (See *calibration*.)

Coefficient of Variation (CV) – A measurement of imprecision (random error), biologic variation, or other variability in a population; mathematically, CV is standard deviation divided by the mean and expressed as a percentage.

Commutability –is the equivalence of results of different measurement procedures using a reference material and representative samples from healthy and diseased individuals.

Comparability testing – Comparison of test results from two or more instruments within the same laboratory or from laboratories at different sites within one health care system that process samples from the same patients. Comparability testing is done to ensure that measurements are similar and can be used interchangeably without causing clinical error. Total allowable error (TE_a) can be used as a basis for judging acceptability of comparability testing results.[4,5]

Control data – Data obtained when one or more quality control material (s)(QCM) is/are measured.

Control charts are graphical displays of control data, plotting time (in days) on the *x*-axis and analyte concentration on the *y*-axis. Control charts are useful for assessing how far away individual data points are from the mean and for spotting drifts (shifts) or trends in results. **Levey-Jennings charts** are a popular type of control chart that use mean ± a multiple of the standard deviation as the control limits (measure of acceptable data).[6]

Control level – "Level" refers to analyte concentration/activity (eg, low, normal, or high) in the QCM. "Running 2 level controls" refers to using two different QCM (eg, one having predominantly normal analyte concentrations/activities and one having predominantly abnormal analyte concentrations/activities) in a given quality control (QC) procedure.

Control limits – The high and low values outside which control data are considered unacceptable ("out-of-control"). For example, in the 1_{3s} rule recommended in these guidelines, control limits are defined as mean ± 3 standard deviations. A single control data point outside the range mean ± 3 standard deviations is said to "violate" the 1_{3s} rule. Use of control rules is sometimes referred to as **statistical QC**.

Control rule – A rule used during analysis of control data to determine whether said control data are acceptable ("in control") or unacceptable ("out-of-control"). Control rules are sometimes referred to as "Westgard Rules".[7] Additional information about control rule nomenclature can be found in other resources.[7,8]

Control run – Measurement of one or more QCM following a specified interval (after a specified number of patient samples, after a specified duration of instrument operation [eg, laboratory shift]).

CV (coefficient of variation) – A measurement of imprecision (random error); mathematically, CV is standard deviation (SD) divided by the mean (mathematical average) and expressed as a percentage:

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

External Quality Assessment (aka external quality assurance, EQA, or proficiency testing, PT) – A program which determines total testing performance by comparing a laboratory's or clinic's test result (including interpretation of results) to a known standard or to an appropriate peer group mean generated from an inter-laboratory comparison in which multiple laboratories measure the same sample using the same test methods, reagents, and controls.[9]

External QC –QC procedures performed by laboratory or veterinary clinic staff that are external to (ie, not built or programmed into) the laboratory instrument. Measuring quality control materials (QCM) is a common example of external QC.

Imprecision (a.k.a. random error or random variation) – Lack of repeatability or reproducibility of the same result; represented by the standard deviation (in units of the test) or coefficient of variation (expressed as percent). (Also see *precision*.)

In-Clinic QC – QC procedures performed by the veterinarian or veterinary staff which include both internal and external QC procedures, such as measurement of quality control materials, participation in an EQA program, and/or comparability testing.

Internal QC –QC functions that are internal to (ie, built and programmed into) laboratory instruments and assess the analytical processes of those instruments.

Instrument performance study – A study performed to characterize an instrument's analytical performance capability, represented by bias (inaccuracy) and imprecision (random error). Instrument performance studies provide data needed for calculation of observed total error (TE_{obs}) and quality control (QC) validation (including ensuring that an instrument can perform to the desired quality requirement). In human laboratory medicine, it is recommended that assessment of imprecision and bias be based on repeat measurement of at least 20 samples.[10,11] This recommendation has been modified to 5 replications for veterinary point-of-care testing.[12,13]

Mean – Mathematical average of values measured.

P_{ed} (probability of error detection) – The "diagnostic sensitivity" of a control rule for detecting analytical error. High P_{ed} means that analytical error is reliably detected; $P_{ed} \geq 90\%$ is recommended in human laboratory medicine.[14] $P_{ed} \geq 85\%$ is recommended as a minimum for veterinary point-of-care testing (POCT).[12,13]

P_{fr} (probability of false rejection) – The "diagnostic specificity" of a control rule for detecting analytical error. Low P_{fr} means that there is a low probability of falsely rejecting control data (ie, of thinking that control data are unacceptable when in fact they do not represent analytical error). $P_{fr} \leq 5\%$ (ie, a diagnostic specificity of > 95%) is recommended in human laboratory medicine and is also recommended for veterinary POCT.[12–14]

POCT (point-of-care test or testing) – Laboratory testing performed outside the traditional clinical pathology laboratory (a.k.a. "reference laboratory").

Precision – Closeness of agreement between independent, repeated results obtained from the same sample under specific conditions. These may be derived in the same day (intraday) or on different days (between or interday).

QA (quality assurance or assessment) – Laboratory procedures that monitor and improve laboratory performance and seek to minimize all types of laboratory error (pre-analytical, analytical, and post-analytical). QA involves quality planning, implementation, monitoring, and assessment, and includes many "common sense" procedures (personnel training, use of standard operating procedures, etc.) routinely utilized in well-run laboratories and clinics.

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 veterinary samples."[15]

QC (quality control) – Laboratory procedures that monitor the analytical performance of instruments and detect error (predominantly analytical). May refer to measurement of quality control materials (QCM) by the instrument operator with subsequent analysis of control data[16] or internal instrument QC functions that monitor analytical processes.

Quality Control Material (QCM) – A 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. An *assayed* QCM is one for which the manufacturer provides expected results for specific instruments or methods. These results include a range and/or mean, standard deviation, and CV. Range may be the mean $\pm$ Z * SD. (Also see definition of Z score.)

Quality control validation – The process of selecting control rules based on a quality requirement, known instrument analytical performance, and desired sensitivity (P_{ed}) and specificity (P_{fr}) for detecting analytical error. QC validation allows robust detection of analytical error because selected rules are *tailored* to the individual instrument and chosen quality requirement. Allowable total error (TE_a) is a commonly used quality requirement.

Quality plan – A concise written statement summarizing the philosophy and framework upon which a facility's quality management program is based.[17]

Quality requirement – A benchmark or standard to which the analytical performance of a laboratory instrument is compared. The quality requirement recommended for POCT in these guidelines is expressed as allowable total error (TE_a).[5]

Standard Deviation (SD) – A measure of variability or diversity associated with random error or imprecision. SD shows how much variation or dispersion there

is 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 the data points are spread over a wide range of values. SD is the square root of a data-set's variance. (Also see *imprecision*.)

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

SOP (standard operating procedure) – A written document that provides information about a process or task. An SOP for laboratory testing may provide a variety of information, but should include detailed instructions for carrying out a laboratory procedure. Use of SOPs helps ensure that laboratory procedures are carried out in a standardized and consistent manner. Suggestions for SOP content can be found in the general ASVCP quality assurance guideline.[15,18]

TE (total error, a.k.a. total analytical error) – The sum of random error (imprecision) and systematic error (bias or inaccuracy). This term may also incorporate other sources of error (eg, some pre-analytical variation, biologic variation, and other factors) that contribute to variation seen in patient results. Total error components that are under direct supervision or control of the laboratory are bias and imprecision.

TE_a (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 insure clinical usefulness.

TE_{obs} (observed or calculated total error) - The sum of measured random error (imprecision) and measured systematic error (bias or inaccuracy). TE_{obs} is defined in this guideline as:

If expressed in units of %,
TE_{obs} = 2CV + absolute bias%

If expressed in analyte units,
TE_{obs} = 2SD + absolute mean difference

TE_{obs} must be calculated for each analyte, is unique to an individual instrument/method, and may vary with analyte concentration or activity. The value 2 is a Z score (see below).

Type I error – False positive or alpha error (see alpha error)

Type 2 error – False negative or beta error (see beta error)

Z score (a.k.a. Z value, normal score, or standard normal deviate) – In statistics, a number indicating how far away an individual value in a dataset is from the mean.[19] The Z score reflects probability of (or confidence in) the TE_{obs} estimate. A Z value of 2 produces roughly a 95% 2-tailed confidence interval for a given estimate.

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