New ASVCP Quality Control Guidelines: How Do They Apply to Your In-House Laboratory

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ASVCP TEa, Point of Care and Proficiency Testing guidelines are pending final approval. These have been written by coauthors including Bente Flatland (University of Tennesse), Kathy Freeman (IDEXX, Wetherby, UK), and Melinda Camus (University of Georgia). The following abstract is based on these documents and represents the most up to date information available.

A VIN survey in 2007 [Bell, 2012] found that >85% of veterinary practices have some form of in-house laboratory which varies significantly from small, one doctor practices to large referral hospitals. Concern about the quality of veterinary in-practice testing has been expressed by veterinarians themselves in published literature[Mitzner 2002, Freeman 1999, Rishiniw 2012]; however, little, if any, concise and practical guidance is available to veterinary practitioners on this topic. Any testing outside of a reference laboratory in human or veterinary medicine is defined as a point-of care analyzer. The goal of this lecture is to provide practical, detailed advice on quality assurance of in-house veterinary equipment that can be used for necessary quality control of an in-house equipment.

Point-of-care analyzers (POCA) are numerous and varied in complexity. POCA can be divided into non-instrumental systems (e.g., reagent test strips); small, hand-held analyzers (e.g., glucometers); and desktop or benchtop analyzers (e.g., automated hematology or chemistry analyzers).Despite the technologic al advances allowing for increasing availability and sophistication of POCA, successful implementation of POCT still requires effective training, organization, and management of staff.

Quality assurance (QA) is the overall program that is put in place to minimize all types of error including prenanalytical, analytical, and postanalytical causes. Quality control (QC) refers to specific steps taken typically to minimize analytical error though QC of sampling and transport as well as reporting of results may also be implemented as needed. The purpose of QA/QC is to prevent production of inaccurate laboratory values that would result in misdiagnosis. In the United States, in human medicine, POCA use is subject to federal regulatory oversight governed by the Clinical Laboratory Improvement Amendments of 1988 (CLIA), no such regulatory oversight is present in veterinary medicine. Additionally, laboratory QA/QC is rarely discussed in veterinary college classes, leaving new graduates with little training in how to establish, evaluate, and maintain the quality of in-practice laboratory testing.

CLIA classifies laboratory tests according to complexity as moderate, high, and waived. This is based on internal quality controls run by the instrument and likelihood of error in diagnostic testing as determined by structured FDA testing. Currently, in the veterinary market the only waived quantitative equipment is the Abaxis chemistry analyzers. All other analyzers should have some form of daily quality controls run by technologists prior to use. This will vary based on analysis and instrument. You should contact technical support for your specific instrument and ask about quality assessment packages offered by your manufacturer. QA/QC packages are offered through IDEXX, Abaxis, and Heska at this time. While technical support (not salesman) at the company is a resource for help with specific machines, knowledge of general principles, as well as external objective opinions should be used to formulate the quality plan.

A quality manual contains detailed policies, standard operating procedures (SOPs), and forms relevant to all aspects of running the laboratory. Only current, approved documents should be in circulation while an archive may be kept for previous policies and instrumentation as needed. The archive should not be in the laboratory but stored in a separate room.

The manual should include

1. SOPs
2. Manufacturer recommendations for maintenance, documented in a written maintenance log. This may include software updates as well as filter, tubing, and other cleaning.
3. QC data for all analysis likely with Levy Jennings charts.
4. Validated reference intervals (see abstract: How To Design Your Quality Assurance Plan)
5. Reagent expiry logs
6. Technician training, continuing education, and competency assessments should be documented and included in the quality manual.

Preanalytical error (sampling, storage, and transport)

Preanalytical error is any potential interferent compound, change in the sample due to environment, storage and transfer prior to actual analysis on the machine. Preanalytical error may result in minor change or cause results that are not interpretable. Samples with moderate to severe hemolysis in general should not be used for interpretation. Please consult your laboratory directly for which analytes may still be used (e.g. PCV) in these situations. While this may make diagnosis of certain disease categories such as IMHA

difficult, it is better to have no data than to have bad data and misdiagnosis. Better to rely on physical exam and clinical assessment in these situations.

For further information about sampling and how to minimize preanalytical error, please see my proceedings on sampling titled: The Cutting Edge: New Molecular and Staining Techniques for Lymphoma and Mast Cell Tumors

Bias (inaccuracy)

Total systematic error which includes constant and proportional bias. Presence of bias may be due to multiple factors. It is the difference between the measured result and the true concentration of 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 the two methods being compared or the average of all the differences between the paired sample values, i.e. the intercept on a Bland Altman graph. Bias may also be expressed as a percentage according to the formula bias(%) = mean target – mean measured/meantarget x 100.

Imprecision (random error)

Lack of repeatability or reproducibility of the same result; represented by the standard deviation (in units of the test) or coefficient of variation (percent). Standard Deviation (SD) is a measure of variability or diversity and shows how much variation or dispersion there is from the average (mean or expected value). A small SD indicates that the data points tend to be very close to the mean, whereas large SD indicates that the data points are spread out over a wide range of values. SD is the square root of a dataset's variance. Coefficient of Variation (CV) is standard deviation divided by the mean and expressed as a percentage. CV for purposes of determination of calculated TE should be determined from precision studies using quality control materials at various levels of analytes, known standards and/or patient samples.

Allowable total error

Westgard was the first to introduce the concept of total error in 1974. 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, i.e. low bias and low CV or SD, respectively. Westgard originally used TE = bias(%) + 1.65CV, but a coefficient (z value) as high as 6 has been used by some authors for method validation studies.

The ASVCP guideline defines observed or calculated total error (TEobs) as bias(%) + 2CV which is consistent with CLIA recommendations. (CLIA '88 Proficiency Testing Limits, U.S. Federal Register). If units of the test are used, then the equation, bias (expressed in units of the test) + 2SD, is used to calculate allowable total error. Total error is the sum of random error (imprecision) and systematic error (bias can be calculated from instrument performance data according to the formula) The calculated TE is specific for a single instrument/method. Allowable total error (TEa) or desirable total error is an analytical quality requirement that sets a limit for both the imprecision (random error) and inaccuracy (systematic error or bias) that are tolerable in a single measurement or single test result to insure clinical usefulness. Tables for allowable total error are found at the end of this document.

Instrument performance evaluation procedure and calculations

The following procedure 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, to ensure adequate ongoing performance, evaluating performance during an external quality assurance (proficiency) program.

Three methods that may be used

1. Comparison with peer group means in an external QA program participation. This typically must be done using an external quality assessment program that is employed to help insure quality laboratory results. While some external quality assurance programs use assayed materials in human medicine, typically in veterinary medicine, unassayed materials are used and there is reliance on the peer group mean. Peer group is defined by same instrument and/or method as that upon which the result is obtained. An external quality assessment using comparison to a peer group is dependent on the numbers of instruments included in the peer group as well as other laboratories' maintenance of equipment and quality control.
2. Comparison with target values provided by manufacturers of assayed quality control materials. An assayed quality control material (known standard) may be repeatedly measured for 5 days to determine mean, bias, SD and coefficient of variation. In this situation, the mean of the results should be compared to the mean of the assayed mean to determine bias. These data can then be used to calculate total error (TEobs) of the analyte. 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. Please consult the manufacturer to insure that it is appropriate for the equipment and methods.
3. Based on comparison with known gold standards for various analytes (standards provided by external regulating or governmental organizations and agencies)

As external quality control programs currently in existence are method specific and methods used by in-clinic laboratories are frequently not represented, option 1 is often not available to the in-clinic laboratory. The following steps are designed for quality assurance assessment for the in-clinic laboratory but may also be used by reference laboratories. 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 carried out using commercially available software programs. Calculations should be performed for each analyte and each QCM.

Measure each QCM daily for a minimum of five days. [Rishniw, 2012] Using these data, for each QCM and each analyte, calculate: Mean (average), Standard deviation (SD), and Coefficient of variation (CV). This value represents between-day (interassay) imprecision of the analyzer. The mean, SD, and CV of the analyzer derived from these QC data are referred to as the calculated or observed SD, and CV.

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

Calculate the analyzer's observed total error (TEobs), using measured CV and measured bias. Compare measured TEobs to TEa. If TEobs < TEa (or very close to it), then the quality requirement is met and instrument is considered suitable for measurement of that analyte. If TEobs > TEa, then several options exist.

Teobs interpretation and assessment of external quality assessment results

Calculated total error (TEobs) for all analytes determined on in-house or reference lab equipment should be compared to the ASVCP total allowable error guidelines found in Table 1. If calculated total allowable error (TEobs) is greater than that which is acceptable (TEa), attempts should be made to identify and correct causes of imprecision (high CV) and inaccuracy (high bias).

If these sources of error cannot be corrected or if problems occur repeatedly, the manufacturer of the instrument and/or a boarded 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, replacement of reagent with new reagent or different manufacturer product, or, potentially, analyzer replacement. Alternately, the initial quality requirements may be relaxed. This is not recommended but is possible only if potential additional error can be tolerated in diagnostic judgment. This requires education of ALL clinicians using the analyzer regarding amended total allowable error of the analyte(s) in question. Any changes outside of the recommended TEa in this document must be justified and documented in a laboratory handbook. This should be done only upon consultation with a boarded veterinary clinical pathologist.

Table 1. Total allowable error as defined in the ASVCP draft guidelines.

Analyte	Low Analyte Values	Within RI	High Values	CLIA Value
Albumin	15%	15%	15%	10%
Alkaline Phosphatase	NCR	25% (20% desirable)	25% (20% desirable)	30%
Alanine Amino Transferase	NCR	20%	20%	20%
Ammonia	NCR	20%	20%	Not found
Amylase	NCR	20%	20%	30%
Aspartate Amino Transferase	NCR	25%	25%	20%
Bicarbonate	20% (15% desirable)	20% (15% desirable)	20% (15% desirable)	10% (RCPA) to 20% (CAP)
Bile Acids	20%	20%	20%	None found
Cholesterol	20%	20%	20%	10%
Chloride	5%	5%	5%	5%
Creatine Kinase	NCR	30%	30%	30%
Creatinine	20%	20%	20%	15%
Gamma Glutamyl Transferase	NCR	20%	20%	15% (RCPA) to 30% (CFX)
Glutamate Dehydrogenase	NCR	30%	25%, >90IU 20%	None found
Glucose	10%	20%	20%	6% Low, 10% High
Iron	30% (15% desired)	30%	30%	20%
Potassium	5%	5%	5%	0.5mmol/L
Lactate	NCR	40%	40%	10 (RCPA) to 30% (CFX)
LDH	NCR	20%	20%	20%
Magnesium	15% desirable, 20% acceptable	15% desirable, 20% acceptable	15% desirable, 20% acceptable	25%
Sodium	5%	5%	5%	4mmol/L
Phosphorus	20%	15%	15%	10-23% (CAP)

Sorbitol Dehydrogenase	NCR	20%	20%	None found
Total Bilirubin	NCR	30% (25% desirable)	30% (25% desirable)	0.4 mg/dl, 20%
Total Calcium	10%	10%	10%	2% (BV) to 8% (CFX)
Total Protein	10%	10%	10%	10%
Triglyceride	NCR	25%	25%	25%
Troponin	NCR	70%	70%	20%CV maximal with around 50% TEa if calculated
Urea	15%	12%	12%	2mg/dl, 9%
Uric acid	10%	10%	10%	17%

Three to five boarded clinicians (ACVIM or ECVIM with various specialties) gave opinions upon clinically desired TEa at low, mid, and high analyte concentrations and activities, except for troponin where the opinion of a single cardiologist was used. Total allowable error was calculated directly from reference equipment used by QALS members using the equation 2CV + bias% = TEa% to insure that TEa was possible. NCR=Not Clinically Relevant. CAP College of American Pathologists Participant Summary, April 2004. CLIA - CLIA '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 27 Sept 2011) and http://www.dgrhoads.com/db2004/ae2004.php (accessed 19 November 2011) 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

Table 1 references

Reed, SM et al. Equine Internal Medicine. 1998. WB Saunders, London.
Hoffman, WE, et al. 1987.. Am J Vet Res 48: 1343.
Mendle, VE. 1988. Am J Vet Res 49: 572
Khanna C, et al.. 1998. JVIM 12:304-309.
Martin LG. Hypercalcemia and Hypermagnesemia. In Advances in Fluid and Electrolyte Disorders/Vet Clinics of North America, May 1998. 28: 565-585.
Cortes YE, Moses L. Compendium. 2007;420-427.
Porciello, F., et al. Austral Vet J, 86: 390–394. 2008.
Moore JN, et al. Equine Vet J 1976;8:49-54.
Lagutchik MS, et al. JVECCS 1998;8:117-127.
de Papp E, et al. JAVMA 1999; 215(1):49-52.
Savage , C. Vet Clin Equine 2008;24:387–40.
Toribio, RE. Vet Clin Equine 2011;27:129–147
Harr KE. Vet Clin Pathol. 2002;31(3):140-151.

References

http://www.westgard.com/assuring-quality-through-total-quality-management.htm (Accessed September, 2012)
http://www.dgrhoads.com/db2004/ae2004.php (Accessed September,2012)
Bell, R., Harr K., Rishniw, et al. Survey of Point of Care Instrumentation, Analysis, and Quality Assurance in Veterinary Practice. Submitted Vet Clin Pathol 2012.
Mitzner BT. It's time for in-house quality assurance (editorial).JAAHA 2002; 38:12-13.
Freeman KP, Evan EW, Lester S. Quality control for in-hospital veterinary laboratory testing. JAVMA 1999; 215: 928-929.
Rishniw M, Pion PD, Maher T. The quality of veterinary in-clinic and reference laboratory biochemical testing. Vet Clin Pathol. 2012;41:92-109.
Westgard JO. Clin Lab Manage Rev 1996 Jul-Aug;10(4):377-81, 384-93, 396-403.
Kenny, D. Scan J of Clin Lab Invest 1999;59:585.
Westgard JO, et al. Clin Chem 1974;20:825-833. 6 2.