# ASVCP Guidelines:
# Quality Assurance for Point-of-Care Testing in Veterinary Medicine

**Version 1.0 (May 2013)**

Developed by the American Society for Veterinary Clinical Pathology (ASVCP) Quality Assurance and Laboratory Standards (QALS) Committee

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**Suggested citation:**
American Society for Veterinary Clinical Pathology (ASVCP). Quality Assurance for Point-of-Care Testing in Veterinary Medicine. Available at http://www.asvcp.org/pubs/qas/index.cfm. Accessed [date].

**Section 1 – Introduction**

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## 1.1 Abbreviations and Definitions

- **ASVCP** –American Society for Veterinary Clinical Pathology
- **CLIA** –Clinical Laboratory Improvement Amendments. The legislation that regulates all laboratory testing (except research) performed on humans in the United States. CLIA is administered by the Center for Medicare and Medicaid Services (CMS) and establishes analytical performance criteria for laboratory testing in human medicine. CLIA divides laboratory instruments according to complexity as "waived", "moderate", or "high".[1]
- **External Quality Assessment (aka external quality assurance, EQA, or proficiency testing)** – A program which determines total testing performance by comparing a laboratory 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.[2]

- **POCT (point-of-care test or testing)** – Laboratory testing performed outside the traditional clinical pathology (i.e., diagnostic or research) laboratory (a.k.a. "reference laboratory"). Synonyms for point of care testing are listed in section 1.2.
- **POCA (point-of-care analyzer or analysis)** – Laboratory instruments and non-instrumental test systems used to perform laboratory testing outside the traditional clinical pathology laboratory.
- **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). See section 2 for additional detail about QA.
- **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."[3]
- **QC (quality control)** –Laboratory procedures that monitor analytical performance of instruments and detect analytical error. See section 2 for additional information about QC.

### 1.2 Point-of-care testing in human medicine vs. veterinary medicine

The term *point-of-care testing* (POCT) broadly refers to any laboratory testing performed outside the conventional reference laboratory and implies close proximity to patients (a.k.a. "bedside", "near-patient", "decentralized", "extra-laboratory", or "in-clinic", testing).[4-6] 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).[6] Potential advantages and disadvantages of POCT are listed in **Table 1**. Despite the technological advances allowing for increasing availability and sophistication of POCA, successful implementation of POCT still requires effective training, organization, and management of staff.[6] POCT should be performed by staff having documented training and competence.[6]

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) and administered by the Centers for Medicare and Medicaid Services (CMS)[1,4]; no such regulatory oversight is present in veterinary medicine. CLIA classifies laboratory tests according to complexity as "moderate", "high", and "waived". Waived tests (non-instrumental testing systems, small, hand-held devices, or more complex instruments having sufficient internal, built-in monitoring) are not subject to regulatory oversight.[1,7] Years ago, POCT fell primarily in the "waived" category. Technological advances and increasing device sophistication mean that, today, POCA can also be found in the other categories.[4,7]

A formalized approach to POCT management, which facilitates regulatory compliance, is common in human hospitals and may include a point-of-care committee, point-of-care coordinator, and/or point-of-care laboratory working group.[5,8] In contrast, a formalized approach is uncommon in academic veterinary teaching hospitals, which vary widely in their approach to POCA management. POCA are commonly purchased and operated by individual hospital units, and input from laboratorians is variable. Similarly, private veterinary practices vary widely in the scope and age of their in-house laboratory equipment and the caliber of their laboratory quality management.

### 1.3 Scope: veterinary point-of-care testing guidelines

Lack of governmental regulation of POCT in veterinary medicine means that veterinarians must demonstrate a commitment to quality assurance (QA) and quality control (QC) from within the profession.[9] Concern about the quality of veterinary in-clinic testing has been expressed by veterinarians themselves in published literature[10,11]; however, little, if any, concise and practical guidance is available to veterinary practitioners on this topic. Veterinary guidelines and textbooks are aimed at laboratory professionals and complex laboratory equipment such as found in reference laboratories. In the authors' experience, laboratory QA/QC instruction in veterinary curricula is scant, leaving new graduates with little training in how to establish, evaluate, and maintain the quality of in-clinic laboratory testing. Acknowledging this void, the Quality and Laboratory Standards (QALS) Committee of the American Society for Veterinary Clinical Pathology (ASVCP) formed a POCT subcommittee in 2009 to provide guidelines for POCT in veterinary medicine. Given the numerous laboratory

tests that can be performed in veterinary practice, the POCT subcommittee excluded from consideration non-instrumental test systems and other tests that CLIA considers waived and focused on small, hand-held and bench-top instruments. An exception was made for glucometers (considered waived by CLIA), which are commonly used in veterinary medicine.

Providing veterinarians with sound, practical advice that may improve the quality of in-house laboratory results has potential to improve the quality of patient care. Implementing a quality assurance program for in-house veterinary laboratories is a risk management strategy with potential to help reduce medical errors. Despite the lack of governmental regulation of POCA in veterinary medicine, veterinarians should not consider themselves immune to liability issues related to the quality of their in-house laboratory testing.

These guidelines are not intended to be all-inclusive; rather, they provide a minimum standard for maintenance of POCA in the veterinary setting. These guidelines are predominantly aimed at bench top hematology and chemistry instruments measuring multiple analytes. Glucometer use will be addressed in a separate ASVCP guideline. It is our hope that these guidelines will provide a practical resource for all veterinary practitioners and veterinary nurses/technicians seeking to improve management of POCA in their particular practice setting. As additional scientific studies become available and POCA analytical performance capability evolves, these guidelines may change; guideline revision is anticipated approximately every 10 years.

### 1.4 Implementation of point-of-care testing: private practice vs. academia

**Figure 1** summarizes the potential use of POCA in veterinary medicine. Whether POCA are used in veterinary private practice, veterinary academia, or the community, QA/QC considerations are similar. Academia offers the advantage of on-site access to board-certified clinical pathologists, who have the greatest expertise in laboratory quality management in our profession, and licensed medical technologists. Academia also offers the option of having a clinical pathology section or laboratory oversee POCA management. Experience in human medicine, based on CLIA surveys of sites performing waived and provider-performed microscopy testing, suggests that involvement of a central laboratory in the oversight of POCT improves the quality of laboratory testing.[4]

Before it is implemented, the need for POCT should be described and justified.[6,12] **Table 2** lists considerations for implementation of POCT. Ideally, the site offering POCT should be allied with a veterinary clinical pathology laboratory or consulting board-certified veterinary clinical pathologist; these can provide advice on various issues, including (but not limited to) device purchase, operator training, results interpretation, trouble-shooting, QA/QC, and health and safety considerations.[6] In human medicine, such relationships may be formalized via a Service Level Agreement between the POCT site and the laboratory.[6,12]

Producing high-quality laboratory results in the veterinary point-of-care setting ideally requires a multifaceted approach combining support of veterinary practitioners by board-certified veterinary clinical pathologists, instrument suppliers, and veterinary technicians trained in principles of laboratory quality management. A recent announcement by the National Association of Veterinary Technicians in America (NAVTA) that it has approved a

clinical pathology specialty for veterinary technicians (Academy of Veterinary Clinical Pathology Technicians) is a very positive development.[13, 14] It seems plausible that busy veterinarians will want to leverage qualified technicians to implement and maintain a quality management program in their practice. Assuming the training includes instruction in the principles of laboratory quality management, employing a veterinary technician having this specialty certification has potential to improve the quality of in-clinic laboratory testing by increasing expertise of the individuals most likely to serve as instrument operators.[15] Technicians having clinical pathology specialty certification might be ideally suited to serve as in-house laboratory supervisors or quality managers. Veterinarians themselves can positively contribute to the quality of in-clinic laboratory testing by recognizing the importance of having a formalized laboratory quality management program within their practice, pursuing continuing education on this topic for themselves and/or their staff, seeking consultation with board-certified clinical pathologists and/or instrument suppliers, and characterizing and monitoring analytical performance of their own, in-house laboratory equipment.[15] Other developments that could facilitate increased quality of in-clinic laboratory testing include development of more external quality assessment (EQA) programs (a.k.a. proficiency testing) aimed at the in-clinic setting, as well as mandating of rigorous standards for maintaining an in-clinic laboratory by veterinary practice accreditation programs.[15] It should be noted that EQA program participation is helpful for scheduled assessment of laboratory testing quality but is not a substitute for daily, in-house QA policies and procedures due to its intermittent nature.

## 1.5 References


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## 1.6 Tables

**Table 1**

**Potential Advantages and Disadvantages of Point-of-Care Testing (POCT)[5,6]**

Material has been adapted from the Medicines & Healthcare products Regulatory Agency (MHRA) Devices Bulletin 2010(02). The MHRA is not responsible for the adapted content.

| Potential Advantages | Potential Disadvantages |
|---|---|
| • Improved turn-around time for results<br>• Potential for improved patient monitoring and care<br>• Smaller sample volumes, may be less invasive<br>• Smaller reagent volumes<br>• Availability outside clinical pathology laboratory's normal business hours<br>• Advantageous in remote areas where rapid access to a clinical pathology laboratory is not possible<br>• May save health care costs if POCT results in shorter hospital stays or reduced hospital admissions | • Poor quality assurance/quality control<br>• Poor quality of analysis due to operator inexperience or errors<br>• Poor record-keeping, poor patient data capture (unless patient data stored by instrument)<br>• Poor cost-capture, lost revenue<br>• Failure to detect erroneous results<br>• Difficulty comparing results with those from centralized laboratory due to different reference intervals or analytical methods<br>• Expense (not same economy of scale as larger clinical pathology laboratory) |

**Table 2**

**Considerations for Implementing Point-of-Care Testing (POCT) in a Veterinary Facility[6, 13]**

Material has been adapted from the Medicines & Healthcare products Regulatory Agency (MHRA) Devices Bulletin 2010(02). The MHRA is not responsible for the adapted content.

## Considerations for Implementing POCT

- What are the hospital's needs regarding laboratory testing (test menus, turn-around time, patient medical needs, pet owner needs, etc.)?
- What patient groups require testing? (Considerations for laboratory testing will differ for a caseload that predominantly consists of emergencies vs. one involving predominantly preventive or elective care.)
- Will POCT enable more rapid diagnosis or treatment in the patient population served?
- How does cost of POCT compare to cost of using a clinical pathology laboratory?
- How will quality of POCT compare to that of using a clinical pathology laboratory?
- Will POCT change hospital service, patient flow, or patient management?
- How easily are POCT patient data and costs captured? Can the POCA interface with the hospital information system or medical records system?
- Does appropriate physical space for housing POCA exist? Is there appropriate storage space for needed reagents, supplies, etc.?
- Who will operate, trouble-shoot, and manage the POCA? Is someone willing to assume responsibility for quality assurance/quality control?
- Are resources available to provide POCA operator training (initial and ongoing)?

**Table 3**

**Potential Areas of Use of POCA in Veterinary Medicine**

| Private Practice | Academic Veterinary Medical Centers | Community | Research Applications |
|---|---|---|---|
| • General practice<br>• Ambulatory practice<br>• Emergency practice<br>• Specialty practice | • Intensive or urgent care units<br>• Field (ambulatory) services<br>• Surgical and anesthesia wards<br>• Internal medicine services<br>• Community practice services & spay-neuter clinics<br>• Avian/Exotics services<br>• Zoo/Wildlife services<br>• Teaching laboratories | • Disaster response teams<br>• Remote area veterinary medicine<br>• Home use by pet owners (e.g., glucometers) | • Pharmaceutical companies<br>• Biotech companies<br>• Academia<br>• Government |

**Section 2 – Quality Assurance Guidelines for Veterinary Point-of-Care Chemistry Testing**

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## 2.1 Abbreviations, Definitions, and Formulae

- **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 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 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

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

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*.)
- **CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS, the National Committee for Clinical Laboratory Standards)** – An organization in human laboratory medicine that writes guidelines and standards. Compliance with CLSI guidelines and standards is voluntary, although CLSI guideline documents are endorsed by regulatory bodies and accrediting agencies.
- **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 $\pm$ a multiple of the standard deviation as the control limits (measure of acceptable data).[6]
- **Control level** – "Level" refers to analyte concentration/activity (e.g., low, normal, or high) in the QCM. "Running two level controls" refers to using two different QCM (e.g., 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"). E.g., in the $1_{3s}$ rule recommended in these guidelines, control limits are defined as mean ± 3 standard deviations. A 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 [e.g., 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:

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

- **External Quality Assessment (a.k.a. external quality assurance, EQA, or proficiency testing)** – A program which determines total testing performance by comparing a laboratory 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 (i.e., not built or programmed into) the laboratory instrument. Measuring quality control materials (QCM) is a common example of external QC.
- **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 (i.e., 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]
- **$P_{ed}$ (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] Ped ≥ 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 (i.e., of thinking that control data are unacceptable when in fact they do not represent analytical error). Pfr ≤ 5% (i.e., 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 (i.e., diagnostic or research, a.k.a. "reference

laboratory"). Synonyms for point of care testing are listed in Section 1 of these guidelines.

- **POCA (point-of-care analyzer or analysis)** – Laboratory instruments and non-instrumental test systems used to perform laboratory testing outside the traditional clinical pathology laboratory.
- **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 +/- 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]
- **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]
- **Standard Deviation (SD)** – A measure of variability or diversity. SD shows how much variation or dispersion there is from the mean (mathematical 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 out over a wide range of values. SD is the square root of a dataset's variance.

- **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 (e.g., 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 + bias%**

  If expressed in analyte units,

  **$TE_{obs}$ = 2SD + 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).

- **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% two-tailed confidence interval for a given estimate.

## 2.2 Taking a formalized approach to veterinary point-of-care testing

Any veterinary hospital possessing laboratory instruments and routinely generating in-house laboratory data is running a clinical pathology laboratory. Veterinary hospitals of all sizes offering in-house laboratory testing should establish a formalized approach to POCT management that includes a written **quality plan or manual**. The quality plan should address the hospital's environment (patient population served, type of testing offered, etc.), facilities, personnel, equipment, and working policies and procedures. The quality plan may be part of a more comprehensive quality manual that also includes detailed policies, chains of command, standard operating procedures, and forms covering all aspects of laboratory function (operational management, analytical, reporting, and QA).[17] It is recommended that all veterinary facilities operating POCA develop and use such documents, and that documents be maintained according to a **document control policy** that ensures only current, approved document copies are in circulation. Recommendations concerning quality documentation can

be found in other resources.[17] **Table 1** summarizes QC and QA options available to veterinary POCA operators.

Academic veterinary medical teaching hospitals and large specialty practices should form a **POCT committee or working group** that oversees POCT policies and POCA acquisition, maintenance, and quality management. All major stakeholders in POCT should be represented in such a body (e.g., clinicians, nurses/technicians, medical records, billing, and information technology). Suggestions regarding composition, responsibilities, and function of POCT committees and working groups are available.[20-22] The individual veterinary facility should decide whether any POCT committee's role is primarily as an advisory body or whether it also has a policing and enforcement role.

### 2.3 Chemistry Instrument Selection

Various analytical methods are used by biochemistry POCA measuring multiple analytes, including reconstituted liquid and dry-chemistry systems and electrochemistry systems. These are reviewed in detail elsewhere.[23] **Factors that may influence selection of a particular chemistry instrument for POCT include:**

- Direct and indirect costs
    - Instrument purchase vs. lease
    - Reagents
        - Closed and open container shelf-lives (is shelf-life practical given anticipated usage?)
        - Liquid (use as is) vs. lyophilized (require reconstitution)

        - Storage requirements
    - Presence or absence of internal instrument QC functions
    - Quality control materials (QCM) used for external QC
        - Availability
        - Cost
        - Closed and open container shelf-lives
        - Liquid (use as is) vs. lyophilized (require reconstitution)
    - Availability and cost of external quality assurance (EQA) programs
    - Cost and length of maintenance/service contracts
    - Time investments
        - Operator training and continuing education
        - Instrument maintenance and trouble-shooting
        - Internal and external QC
        - Turnaround time/throughput capability
        - Maintaining inventory
- Type of testing (testing of individual analytes vs. testing of multiple analytes via cartridges, rotors, slides, or strips)
- Sample volume and requirements (plasma, serum or whole blood)
- Instrument
    - Size (“footprint”)
    - Ease of instrument and software operation
    - Ambulatory capability, if needed

- Speed of sample processing
- Environmental requirements (temperature, humidity, vibration)
- Data management
  - Report format
  - Data storage and recall
  - Flags: operational, abnormal values, etc.
  - Presentation and archiving of control data (e.g., can the instrument create control charts?)
- Maintenance requirements
- Analytical performance (minimum performance specifications may be available from the instrument manufacturer)

- Health and safety considerations
  - Waste generated: amount, type, and packaging/disposal required
  - Infection control
- Customer support
  - Training and continuing education for instrument operators
  - Technical service provided by the manufacturer
- Instrument reputation
  - Feedback from other instrument users
  - Analytical performance data reported in medical literature

**2.4 Personnel (equipment operator) training and assessment**

Adequate equipment operator training is an essential component of QA and generation of accurate laboratory results.[18] The hospital manager should ensure that all personnel performing laboratory testing are properly trained, and provision should be made for both initial training and continuing education. **Examination audits** (competency assessments) should be carried out by the manager (or other qualified individual) to document competence. Audits should follow initial training and performed periodically thereafter at the manager's discretion. Maintenance of written operator training logs or other training records is also recommended.[15] Audits and logs should be archived such that retrospective evaluation is possible; relevant state, national, and professional accreditation requirements should be met.

**2.5 Instrument maintenance**

Manufacturer's recommendations for maintenance and cleaning of equipment should be followed and documented. With chemistry instruments, the light source should be checked regularly according to manufacturer's instructions to ensure that deterioration, which could result in erroneous results, is not present. The light source should be replaced as needed. Periodic software updates should be performed as needed and recommended by the manufacturer. **Instrument performance studies (see section 2.7) should be carried out immediately following instrument purchase/set-up and a period of operator familiarization, but before the instrument is used routinely to evaluate patient samples. Follow-up instrument performance studies are recommended at a minimum annually thereafter (more often as needed) to ensure that analytical performance does not deteriorate with instrument aging or other events in the life of the instrument that could influence analytical performance**

**(parts replacement, software upgrades, etc.)**. An **instrument log** should be maintained and kept near the instrument to document any problems with the POCA or its results, any trouble-shooting performed, and any corrective action(s) taken as a result. Efficacy of corrective actions should be confirmed in writing archived in the instrument log.[15]

## 2.6 Quality control and quality assurance options for point-of-care testing

### *2.6.1. Internal instrument quality control functions*

Some POCA have built-in, internal QC functions. Such functions may include QC samples, measuring system function checks, electronic system checks, and calibration checks.[24] Internal instrument QC functions provide important data that should be reviewed regularly and that may be used for trouble-shooting aberrant laboratory results. Importantly, veterinarians must realize that internal instrument QC functions monitor only certain aspects of the testing process and do not simultaneously assess the entire analytical system (instrument, reagents, and operator).[25, 26] Most often, it is the operator that is not assessed by these functions. Assessment of reagent variables by internal QC functions varies by instrument. Internal instrument QC functions should not be considered a substitute for the external QC options discussed below, but should be used in addition to them.[25, 26]

### *2.6.2 External quality control ("running controls")*

#### *2.6.2.1 Type and number of quality control materials (QCM) for use in instrument performance studies and external quality control*

The best way to determine whether a laboratory instrument is performing adequately is to measure material having known analyte concentrations/activities.[16] Materials having known concentrations/activities include calibrators and **quality control materials** (**QCM**). Use of QCM is the only way to confirm proper function of the entire POCA system, including instrument, reagents, and operator.

QCM are available through biochemistry supply companies worldwide and may be designated as assayed or non-assayed materials.[27] Use of assayed QCM is recommended. For assayed QCMs, the manufacturer reports a mean analyte concentration (frequently with a range and standard deviation) for each analyte in the QCM package insert, together with relevant analytical methods used.[27] Veterinarians should ideally purchase QCM having a mean determined by the same analytical method(s) as used by the POCA in question. Manufacturers of the QCM should supply information regarding compatibility and use of their product with specific instruments, and manufacturer's recommendations should be followed. All commercially available QCM have a lot and/or batch number and an expiration date. QCM degrade over time, and expiration dates must be observed. QCM should be stored and handled appropriately, as directed by the manufacturer.[26]

Using a minimum of two levels of QCM (normal and one abnormal) is recommended for all instrument performance studies, including external quality assurance and proficiency testing.[13] Using three levels of QCM (low, normal, and high) may be preferred during in-clinic instrument performance evaluation (e.g., at instrument purchase and set-up or during annual performance reevaluation) to demonstrate performance over a wider range of medically relevant values. Routine daily monitoring of instrument performance (routine "running of

controls") should also be performed with at least one level of QCM following instrument set-up and initial performance evaluation.[12, 13] Whether a normal or an abnormal control (or both) is used for daily running of controls may depend upon instrument type and the patient population served; instrument manufacturer recommendations should also be followed. Optimally, consultation with a QC specialist and QC validation should be used to determine the number of QCM levels used (typically one, two, or three). Justification for the number of QCM used during routine monitoring should be documented in the instrument log.

*2.6.2.2 Options for interpreting control data*

QC data should be recorded and archived for two years or as required by law.[28] Options for interpreting control data are discussed below.

*2.6.2.2.1 Interpreting control data without validated control rules*

Control data should always be inspected for instrument error flags, and flagged results should be investigated and control runs repeated as needed. Similarly, control data for each analyte should always be compared to the QCM manufacturer's assayed range; data outside the assayed range likely reflect egregious analytical error and should be investigated. However, manufacturer's reported ranges may be wide, and using the QCM manufacturer's assayed range as the *only* control limits during interpretation of control data is *not recommended*. When using the QCM manufacturer's assayed range as the only control limits, it is possible that excessive analytical error (based on a quality requirement) for a particular POCA could occur and yet control results could still be within the QCM manufacturer's reported range. In other

words, using QCM manufacturer's assayed ranges as the only control limits is insensitive and may allow clinically significant analytical error to go undetected. Rather, control data should be interpreted in light of a quality requirement, knowledge of the given POCA's analytical performance capability (represented by $TE_{obs}$), and adequate sensitivity and specificity for detecting analytical error (i.e., using validated control rules – see section 2.6.2.2.2).

Control data may be graphed for visual inspection of trends over time. **Control charts** are useful for spotting marked deviations from the mean as well as data drifts (shifts) over time that should prompt investigation of POCA function.

*2.6.2.2.2 Interpretation of control data using validated control rules*

Interpretation of control data using validated rules (statistical QC) is the most sensitive and specific way to detect analytical error. Sensitivity (probability of error detection, $P_{ed}$) and specificity (probability of false rejection, $P_{fr}$) of control rules for detecting analytical error hinge on three factors: *the quality requirement* (e.g., allowable total error [$TE_a$]), *which control rule(s) is/are selected*, and *how many levels of QCM are analyzed* each time controls are run. Clearly, running two levels of QCM each time controls are run yields more data about analyzer performance than running only one level of QCM. However, to minimize the cost and time involved, using fewer levels of QCM may be preferred, assuming that less data can provide enough information to detect analytical error with reasonable certainty.

How are control rules selected and the number of QCM levels decided upon? Broadly speaking, **QC validation** has three phases (also see section 2.7): for a given instrument, for each analyte measured, (1) choose a quality requirement ($TE_a$ is recommended for POCA, and

recommendations are available from ASVCP[5]), (2) do an instrument performance study (section 2.7) and calculate $TE_{obs}$, and (3) if analytical performance is acceptable ($TE_{obs} < TE_a$), choose an appropriate control rule and number of QCM levels. Selecting appropriate control rules requires QC validation tools (e.g., commercially available software[29], specially designed tables[12], or specially designed charts[30]); consultation with a QC specialist is ideally recommended. **The control rule called $1_{3s}$ is recommended for POCA; this rule states that a control data point is considered unacceptable ("out-of-control") if it falls outside the range of $\pm$ 3 SD from the mean.** Any one data point outside $\pm$ 3 SD is a rule "violation", leads to "rejection" of that QC run, and should prompt trouble-shooting of POCA function.[12, 13] Once any instrument malfunction has been corrected, QCM should be measured again once. Patient samples should not be measured until repeat analysis of QCM demonstrates "in control" results for all analytes. All corrective actions should be documented in the POCA's instrument log. **Using one or two levels of QCM is recommended for POCA.** Veterinary practitioners may use **Table 2** (modified from Rishniw, et al.[12]) to include $TE_a$ values currently recommended by ASVCP) to determine if analytical performance of their own particular POCA is robust enough such that $1_{3s}$ and one or two levels of QCM may be used with the recommended sensitivity and specificity.

What are the recommended sensitivity (Ped) and specificity (Pfr) for detecting analytical error during QC of POCA? **It is recommended that the $1_{3s}$ rule detect analytical error with $P_{ed} \geq 85\%$ and a $P_{fr} \leq 5\%$.**[12-14] This level of error detection means that, during routine QC, the $1_{3s}$ rule has a > 85% chance of detecting analytical error and a < 5% chance of falsely rejecting control data that are, in fact, acceptable. How successfully the $1_{3s}$ rule performs for a given analyte and a given POCA, and whether one or two levels of QCM are needed, is dictated by

that POCA's analytical performance. For instruments having good analytical performance, only one QCM level will be needed; for those with less good performance, two levels of QCM will be needed (or analytes may not be "QC-able" at all).

During QC validation, a crucial question for chemistry instruments measuring multiple analytes is **"how many analytes are 'QC-able' using $1_{3s}$?"** That is, for how many of the measured analytes can $1_{3s}$ be applied with the desired sensitivity and specificity for detecting analytical error ($P_{ed} \geq 85\%$ and a $P_{fr} \leq 5\%$)? **This question should be investigated using both one and two levels of QCM.** If one level of QCM provides desired $P_{ed}$ and $P_{fr}$, stop there. If not, investigate whether two levels of QCM will provide adequate error detection. **A POCA does not "qualify" for statistical QC (i.e., statistical QC should not be performed) if the $1_{3s}$ rule cannot provide $P_{ed} \geq 85\%$ and a $P_{fr} \leq 5\%$ for > 75% of measured analytes using 2 levels of QCM.** While it is true that other candidate control rules could be evaluated in this situation, it is likely that other statistical solutions will not be easy or cost-effective. Therefore, **if statistical QC using $1_{3s}$ is not possible at the recommended $P_{ed}$ and $P_{fr}$ for at least 75% of measured analytes and instrument analytical performance cannot be improved** (based on consultation with the manufacturer ± a QC specialist)**, then instrument replacement should be considered.**

*2.6.2.3 How often should controls be run?*

The longer the interval between control runs, the more difficult it is to detect trends or shifts in instrument analytical performance. This is particularly true for low-volume laboratory settings evaluating few patient samples, since, in those settings, there are not sufficient patient data to help detect abnormal trends and shifts. Each hospital or clinic must ponder the "cost"

(actual financial costs of repeating samples, potential liability of making medical decisions using poor quality laboratory data, costs to client relations, etc.) of infrequent QC and managing laboratory results generated between an acceptable and an unacceptable QC event. Clinics operating laboratory instruments connected to a laboratory information system may be able to work with instrument manufacturers to receive QC services and feedback via remotely monitored patient and/or control data.[31]

*2.6.2.3.1 The dilemma presented by unit devices*

Recommendations for QC frequency of POCA is complicated by the fact that many POCA (particularly instruments measuring biochemical analytes) utilize single-use, disposable cartridges, cassettes, rotors, slides, or strips. These "unit devices" vary in complexity and may contain electrodes, microfluidic networks, reagents, and/or mechanisms for separating or aliquotting samples.[32] POCA using unit devices also vary in complexity and may or may not contain pipettes or tubing that could be subject to malfunction (plugs, leaks, etc.). A single POCA may utilize multiple unit devices measuring different analytes (e.g., cartridges, cassettes, rotors, or slides offering different "profiles" or "panels"). Such devices present several dilemmas:

- If external QC is done, a unit device must be used for each QC run, adding to the overall total cost of laboratory testing.
- If external QC is done, a QC run only evaluates quality of that one particular unit device. If resulting control data are acceptable ("in-control"), it is assumed that other unit devices from the same lot have similar quality and are also appropriate for patient use.

This is generally true; however, quality can vary from unit device to unit device, even within the same lot.

- Unit devices may or may not contain internal QC functions that assess reagents or other components of the analytical process. Information about efficacy of such internal QC functions may not be available if it is considered proprietary by the manufacturer.

Making recommendations concerning necessity and frequency of external QC for unit use POCT devices ideally requires risk assessment on an individual clinic or laboratory basis (see below). Such devices therefore require monitoring via external quality assurance (proficiency testing) program participation.

*2.6.2.3.2 Frequency recommendations*

Daily analysis of QCM (i.e., at least every 24 hours, or each day an instrument is to be used for patient samples) is recommended by most laboratorians.[33] This recommendation was made specifically for larger, more complex laboratory analyzers using liquid reagents.

Some POCA used in human medicine are considered "waived" under CLIA[34]; some of these POCA have analogues in the veterinary market. Waived tests are not subject to CLIA analytical performance requirements; following manufacturer instructions for device operation is sufficient for regulatory compliance. For more complex POCT systems, presence of internal QC functions (with testing and documentation of efficacy by the instrument manufacturer) is required to achieve waived status. POCA manufacturer recommendations for frequency of analyzing QCM vary widely and may be as infrequent as monthly, may be based on the volume of testing, or may be based on changes in unit device lot numbers. The more frequent the

monitoring of instruments, the greater the likelihood that analytical error is detected before erroneous patient results are reported.[24] In the authors' experience, QC intervals greater than weekly do not provide adequate control data.

Can a blanket recommendation for external QC of POCA (which may be waived or non-waived) be made? The Center for Medicare and Medicaid Service (CMS, which administers CLIA), currently advocates a risk-based approach to laboratory quality management for all measuring systems and is phasing in a new risk assessment-based guideline developed concurrently by CMS and CLSI.[24, 35] Components of risk assessment that are considered under this new guideline are numerous and require familiarity with more complex QC ad QA procedures in order to assess probability of error occurrence, clinical impact of error, and cost of risk mitigation. A formalized, risk assessment-based approach to quality management is currently uncommon in veterinary laboratories and clinics, but should be considered and could be used to tailor the general recommendations given below. Consultation with a QC specialist may be of benefit in helping assess risks if this approach is used. **The following general QC frequency is recommended for veterinary POCA:**

<u>For all instruments</u>:

- Daily monitoring of any internal instrument QC functions is recommended. Information regarding efficacy and monitoring capability of internal QC functions may be available from the instrument manufacturer, and instrument operators should be familiar with these.
- Instrument error flags should always be investigated.

- It is not necessary to perform QC on days the instrument will not be used for patient samples (i.e., days the clinic is closed for business or on any other days when patient samples are not measured).

<u>For instruments using unit devices (single-use disposable cartridges, cassettes, rotors, slides, or strips that contain bar code tracking of the unit device)</u>:

- Unit devices for some POCA include internal QC functions that evaluate components of the analytical process (so-called "integrated QC samples"[24]) and, in some instruments, reagents. Information regarding what is monitored by such integrated QC samples (and efficacy of such monitoring) may be available from the instrument manufacturer, and instrument operators should be familiar with these. For instruments using such unit devices, daily external QC may not be required. External QC should be performed when:
    - A new lot number of unit devices is opened (or when a new shipment of unit devices is received, even if these are of the same lot number)
    - The instrument undergoes maintenance, calibration, software upgrades, or any other manipulation by the manufacturer that influences analytical performance
    - Suspect or aberrant patient data are observed and investigation of instrument performance is needed
    - Weekly external QC should be considered to help document ongoing stable performance and provide sufficient control data to detect trends or shifts suggestive of problems and to provide familiarity with performance of control material(s) used for trouble-shooting suspect or aberrant patient results.

- If unit devices do not include internal QC functions that assess reagent, daily external QC using at least one level of QCM is recommended (see section 2.6.2.2.2 for additional discussion about number of QCM levels).

For instruments without unit devices:

- Daily external QC using at least one level of QCM is recommended (see section 2.6.2.2.2 for additional discussion about number of QCM levels).

While daily external QC is the ideal recommendation, actual QC frequency may be tailored to the individual clinic setting based on the estimated risk of error occurring, instrument analytical performance capability, stability of analytical performance over time, and consultation with a QC specialist. Justification for less than daily external QC frequency should be documented in the instrument log and relevant SOPs.

*2.6.2.4 Changing quality control material lot numbers*

If control data are being monitored using control charts (section 2.6.2.2.1) or control rule(s) (section 2.6.2.2.2), then the control limits used to ascertain whether control data are acceptable should be derived from the mean and standard deviation (SD) of the control data. QCM from different lots may not have the exact same analyte concentrations, although these should be close. This issue impacts QC because changing QCM lots (and thus analyte concentrations in the QCM) alters the control limits (by altering mean and SD of the data) used to decide if QC data are "in-control" or "out-of-control".[36, 37] Instrument recalibration (as may

occur with software updates and other adjustments) also impacts QC, because recalibration may alter how the instrument measures and may impact mean and SD of the control data.

Obviously, the longer one lot of QCM can be used, the less frequently control limits must be recalculated. Ideally, a clinic or laboratory should purchase (or reserve with the manufacturer, based on estimated needs over the course of a year) enough of one QCM lot to last for an entire year. This may or may not be possible, depending upon stability of the QCM and manufacturer production schedules. Chemistry QCM lots may be available for 12 months or longer. If QCM lots are changed only once per year, recalculation of control limits (which requires repeat measurement of QCM) can be combined with the annual reassessment of instrument performance capability (sections 2.5 and 2.7). **The following steps are recommended when a QCM lot change occurs (or when an instrument is recalibrated) and are applicable to assayed and non-assayed QCM:**[12, 27, 36]

Carry out these steps *before* completely running out of the old QCM lot! Sufficient old QCM lot is required to allow the overlap described below.

↓

Analyze old and new QCM lots concurrently on 5 consecutive days (i.e., until 5 data points are accumulated for both materials). During these 5 days, use results from the old QCM lot to determine whether analyzer performance is "in control" or "out-of-control".

↓

Calculate mean of the <u>new</u> QCM lot data.

↓

Calculate SD of the <u>old</u> QCM lot data based on the most recent 30 days of use (including the 5 days referred to above).

↓

Use these two values to calculate the new control limits. E.g., if the $1_{3s}$ control rule is being used, then the new control limits would be

$$\text{High control limit}_{new} = \text{Mean}_{\text{new QCM lot}} + 3\text{SD}_{\text{old QCM lot}}$$

$$\text{Low control limit}_{new} = \text{Mean}_{\text{new QCM lot}} - 3\text{SD}_{\text{old QCM lot}}$$

↓

Once at least 5 days' worth of data (or up to 20 days) from the new QCM lot has been accumulated, recalculate the control limits using mean and SD from those data:

$$\text{High control limit}_{new} = \text{Mean}_{new\ QCM\ lot} + 3\text{SD}_{new\ QCM\ lot}$$

$$\text{Low control limit}_{new} = \text{Mean}_{new\ QCM\ lot} - 3\text{SD}_{new\ QCM\ lot}$$

↓

Continue to use these control limits until the QCM lot number changes again.

↓

Repeat as needed.

*2.6.3 External quality assessment programs (a.k.a. external quality assurance, proficiency testing)*

In addition to regular in-clinic QC, participation in an external quality assessment (EQA) program is recommended for all equipment, including CLIA waived instruments, to ensure quality of POCT results. Prior to participation in an EQA program, the veterinarian should inquire whether other facilities using the same POCA and/or method are participating. This ensures that an appropriate peer group is available within the program for comparison. At least quarterly (periodic) participation is recommended; less frequent participation is unlikely to yield useful data. A limitation of this recommendation for POCA operators is that most current EQA programs available to veterinarians (and supplying veterinary samples) are targeted at reference laboratories, and an appropriate peer group may be difficult to find for POCT. More EQA programs aimed at veterinary POCT and veterinary in-clinic laboratories are needed.

"Acceptable" POCA performance relative to EQA results is defined as POCA results within $\pm$ $TE_a$ of the peer group (or assayed) mean or within $\pm$ 2SD of the peer group (or assayed) mean, whichever is more stringent.[5, 38] If POCA performance is not acceptable based on EQA

results, then investigation to determine if this represents random error or another problem should be undertaken. Such investigation may include:

- Review of control data for several weeks immediately prior to, during, and following the time of EQA results.
- Review of patient results for several weeks immediately prior to, during, and following the time of EQA results
- Comparison of current results with a facility's previous and/or future performance in the EQA program
- Consultation with the POCA manufacturer's technical representative or a QC specialist

If corrective actions were taken as a result of unacceptable performance, then it is important to determine and document if these actions were effective in returning performance to an acceptable level based on future EQA results.

*2.6.4 Comparability Testing*

In human medicine, "comparability" refers to agreement between patient results for an analyte using different measurement procedures (different instruments and/or methods) within one health care system.[4] In this document, the term "comparability testing" is used to refer to agreement between results as measured at different testing sites (e.g., veterinary clinic and reference laboratory). Timing of comparability testing can be frequent (e.g., daily, weekly), periodic (e.g., quarterly, biannually), or "special cause" testing. Special cause testing is performed in response to an alert from a QC procedure or other triggering event.[4] In veterinary

medicine, a common scenario initiating special cause comparability testing is the desire to check an unexpected or aberrant patient result from an in-clinic analyzer by sending an aliquot of that patient's sample to a reference laboratory.

Regularly scheduled frequent or periodic comparability testing (monthly or quarterly) using a stable patient sample or QCM potentially could be used by a veterinary clinic to monitor analytical performance of its POCA. In comparability testing, it is highly likely that results from two different analytical methods are being compared (e.g., dry-chemistry POCA at the veterinary clinic and wet-chemistry analyzer at the reference lab), which complicates interpretation of results. A simple subjective comparison of results may be sufficient (e.g., are both results within their respective reference intervals or above or below the respective reference intervals by a similar degree?). A more objective comparison involves using POCA results $\pm$ $TE_a$ as an "acceptance interval" for comparing POCA and reference laboratory results.[5, 12] If reference laboratory results fall within the interval defined by POCA results $\pm$ $TE_a$, then results from the two methods are considered comparable.[5] If the reference laboratory result does not fall within the POCA +/-$TE_a$ interval, then careful consideration of both results and trouble-shooting of the POCA are needed.

*2.6.5 Non-statistical quality assurance procedures*

**Non-statistical QA** is an essential component of veterinary laboratory quality systems, particularly given the variety of species, physiologic differences, and disease manifestations that veterinarians routinely encounter.[17] All veterinary laboratories, regardless of their size or complexity, should have non-statistical QA procedures in place; implementation of non-

statistical QA procedures is vital to ensure laboratory result quality. Recommended non-statistical QA procedures in veterinary medicine include:[15, 17]

- *Use of written policies, SOPs, and forms*, as described in section 2.2
- *Documentation of personnel training*, as described in section 2.3
- *Use of non-expired, properly stored and handled reagents and QCM.* If reagents are frozen, these should only be stored in non-self-defrosting freezers; exposure of frozen reagents to the repeated partial or complete freeze/thaw cycles of self-defrosting freezers may cause premature degradation.[4]
- *Documentation of instrument maintenance and repairs*, as described in section 2.4
- *Regular monitoring (and maintenance, if applicable) of*
    - Water and electrical power
    - Temperature of refrigerators, freezers, and water baths
    - Pipettes, centrifuges, balances, and timers
- *Blood smear review* – Review of a well-made blood smear and correlation with numerical results reported by automated hematology analyzers (see section 3 for additional detail and other recommendations applicable to hematology testing specifically).
- *Repeat criteria* – Used to determine when a laboratory test should be repeated in order to eliminate random error as the cause for an unusual or unexpected result. Life-threatening results or those considered incompatible with life (physiologically improbable) represent the minimum results that should be repeated.

- *Medical review criteria* – Describe laboratory results requiring immediate attention of a veterinarian and for which further action (e.g. repeat testing or verification by sending out to a reference laboratory) may be required.
- *Monitoring patient data* – Unexpected, aberrant results from individual patients may help identify random error, while review of data from multiple patients over time may help identify systematic error (e.g., drift). A full discussion of using patient data to monitor laboratory quality is beyond the scope of these guidelines, and the interested reader is referred to other resources for additional detail.[6, 31, 38, 39]
    - Common sense dictates that data from any patient is assessed to ensure it falls within physiologic limits compatible with life (also see repeat criteria, above). Data from individual sick patients should be evaluated to ensure that these make sense, given other clinical information (physical examination, other laboratory data, imaging data, etc.).
    - Obvious data drifts (e.g., results for a particular analyte from multiple patients that are trending upwards over time in data) should be investigated, as this likely represents egregious systematic error (e.g., a decaying lamp or reagent, calibration problem, etc.).

Interpretation of unexpected, abnormal laboratory data in clinically healthy patients presents a challenge. Knowledge of test result patterns and implementation of repeat criteria and medical review criteria are important non-statistical QA measures that may help veterinarians judge the significance of abnormal laboratory results in apparently healthy animals. In some cases, repeat testing on a second specimen may be needed to demonstrate the persistence of an

abnormal finding. The degree of abnormality in the result and the likelihood of clinical significance (in the clinician's estimation) may also be important in determining if further investigation or continued monitoring should be undertaken.

## 2.7 Instrument performance study and QC validation steps

This section provides additional detail regarding instrument performance evaluation and QC validation as referred to in section 2.6.2.2.2, above. It is recommended that veterinary practitioners consult with a QC specialist as they carry out steps outlined in this section.

### *2.7.1 Measuring imprecision and bias*

Instrument analytical performance capability is represented by imprecision (random error), bias (inaccuracy), and observed total error ($TE_{obs}$). Imprecision and bias are measured during an instrument performance study carried out by the clinic using a minimum of two levels of QCM; this must be done before it can be decided whether the $1_{3s}$ rule is appropriate for monitoring control data from the POCA. Veterinarians should be aware that analytical performance of one instrument measuring multiple analytes may vary from analyte to analyte, and that analytical performance may differ from instrument to instrument, even among instruments of the same make and model.[12] **Instrument performance study steps (formulae are found in section 2.1 under each respective definition):**

1. Measure each assayed QCM daily for a minimum of five days[12, 13], or up to 20 days.[10, 11] From these data, for each QCM and each analyte, calculate (commercially available spreadsheet programs can do these calculations):

a. Mean (average)

b. Standard deviation (SD)

c. Coefficient of variation (CV)

2. Calculate the analyzer's bias. Use the formula for expressing bias in units of %. The measured mean is the mean from step 1.a, above. The target mean is the QCM manufacturer's reported mean (using the same instrument and/or method as the POCA in question). QCM manufacturer's reported means are commonly found in the QCM package insert and are categorized according to the instrument and method producing the assayed values. **Note:** 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.
3. Calculate the instrument's observed total error ($TE_{obs}$).

### *2.7.2 Comparing $TE_{obs}$ to $TE_a$ and confirming applicability of $1_{3s}$*

If $TE_{obs} < TE_a$ (or very close to it), then the quality requirement is met and instrument is considered suitable for measurement of that analyte. If $TE_{obs} > TE_a$, then options include (1) improve POCA performance for measuring those analytes, if possible, (2) instrument replacement, or (3) monitoring control and/or patient data for those analytes using other means (such as EQA program data, comparability testing, and/or non-statistical QA procedures).[12, 13] A possible fourth option is to consider "relaxing" the $TE_a$ such that $TE_{obs} < TE_a$[13]; however, this is not generally recommended. If altering $TE_a$ is considered, a QC expert

should be consulted. Any alterations in $TE_a$ (including the justification for doing so) should be clearly documented in the POCA's instrument log and relevant SOP's.[5]

If $TE_{obs} < TE_a$, use Table 2 (or another QC validation tool) to confirm that the $1_{3s}$ control rule can be used to monitor each analyte with the recommended sensitivity and specificity for detecting analytical error (also see section 2.6.2.2.2). Table 2 is an appropriate starting place for QC validation, and additional QC validation tools can be employed as needed. If a "solution" cannot be found using Table 2 (this can be confirmed by a QC specialist using QC validation software), then $1_{3s}$ should not be used because it will not provide the recommended $P_{ed}$ and $P_{fr}$ (i.e., statistical QC using $1_{3s}$ will not be sufficiently accurate or cost effective). To date, scientific study of $1_{3s}$ rule performance has only been carried out with chemistry analyzers.[12] Presumably, $1_{3s}$ can be applied with similar success to other types of POCA (e.g., hematology analyzers) assuming analytical performance is robust enough; however, additional studies are needed to confirm this. Pending such studies, $1_{3s}$ is recommended as the first candidate control rule for all POCA analytes whose $TE_{obs} < TE_a$.

## 2.8 Reference intervals

Establishing (or validating) reference intervals is a critical component of instrument and method validation, and assays not having reference intervals are considered not fully validated in any setting. Manufacturers may provide reference intervals for POCA patient data interpretation or these may be generated *de novo* by the clinic or laboratory. **When reference intervals are transferred from an instrument manufacturer or from other clinic or laboratory using an instrument of same or similar analytical methodology, validation of reference**

**intervals is required.** Whether a reference intervals is suitable for transference and validation should be based on assessment of the reference sample population and target clinic or laboratory's patient population and statistical comparison as defined in the ASVCP RI Guidelines.[40, 41] If transference is appropriate, then validation can be pursued using 20 patient samples as detailed in the reference interval guideline.[40, 41] Reference intervals and appropriate units for each analyte should be listed with patient data in a laboratory report in the patient's medical record.

### 2.9 Patient results reporting

If patient results (instrument print-outs or electronic data) are not pasted directly into a paper medical record or transferred electronically (downloaded) to a computerized hospital information system, then a system should exist to verify accuracy of transcribed results. Corrected results should be clearly identified, in the event that a reported result is revised. Manually entered (handwritten or typed) annotations should be initialed and dated. Information concerning sample characteristics (e.g., lipemia, hemolysis, or other discoloration) should be included with patient results. If there is potential for interference based on POCA manufacturer information (e.g., user manual), then affected results should be highlighted or flagged in some way (if this is not already done automatically by the instrument).

Archiving and backup of electronic patient data must exist to insure integrity over time as required by law. Additionally, paper documentation of patient data must use ink that will last for the legally required duration. Carbon-burned print produced by some instrument printers will fade and is not adequate for patient data archiving.

## 2.10 Summary

A formalized and comprehensive approach to the quality management of POCA is recommended in all veterinary settings, and it is the ASVCP's recommendation that private veterinary practitioners operating in-house laboratory equipment should implement a comprehensive laboratory quality management program. Facilities should document laboratory quality procedures by means of a written quality plan or manual that includes policies, SOPs, and forms. Logs and records (e.g., instrument performance logs, instrument maintenance logs, and operator training logs) and audit results (e.g., of operator examination audits) should be maintained and archived. Equipment operator training and assessment should be documented and ongoing. Internal instrument QC functions, external QC procedures (measurement of QCM and interpretation of control data, participation in an EQA program, comparability testing), and non-statistical QA procedures should be routinely used. Reference intervals for patient data interpretation should ideally be validated, and measures should be in place to ensure accurate patient results reporting.

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## 2.12 Tables

*Table 1 Quality Assurance Options for Point-of-Care Analzyers (POCA) in Veterinary Medicine*

| QA Method | Explanation or Examples | Strengths | Consideration(s) |
|---|---|---|---|
| **Internal QC** | | | |
| Internal Instrument QC Functions | QC functions built and programmed into the instrument | Easy to use. Do not require special training in QC. | • Only evaluate certain analytical functions of the instrument and do not assess the entire measurement system. Most often, performance of reagents and operator's skill are not assessed.<br>• Require operator to understand the significance of the function check and to investigate any flagged results or abnormalities identified by these. |
| **External QC** | | | |
| QC without tailored statistical control rules (without QC validation) | Use of QCM to generate control data; analysis of control data in light of QCM manufacturer target values with or without Levey-Jennings control charts | Provides unknown Ped and Pfr. May detect egregious errors in POCA function. | • QCM must be purchased.<br>• Control data must be recorded and analyzed.<br>• Manufacturer target values may or may not be generated using the same analytical method as the POCA in question.<br>• Data analysis does not take into account specific POCA analytical performance or a quality requirement.<br>• Required QCM and reagents add expense, particularly if "out-of-control" results are repeated. |

| | | | |
|---|---|---|---|
| Statistical QC using tailored control rules (QC validation) | Use of QCM to generate control data; analysis of control data in light of a quality requirement and selectively chosen statistical "control rules". | Provides excellent assessment of whether analytical error is present if:<br>• Control rule chosen has high Ped and low Pfr<br>• Based on actual performance of individual POCA | • QCM must be purchased.<br>• Control data must be recorded and analyzed.<br>• QC validation and data analysis requires training in QC.<br>• Required QCM and reagents add expense. |
| EQA programs | Program administered by an independent agency that sends samples (patient samples or assayed quality control material) for analysis to participating facilities. Results are compiled by the program administrator and reported to program participants. | Allows comparison of one's results with that of other clinics and laboratories using the same or similar instruments or methods assuming an appropriate "peer group" is available. | • Peer group availability may limit comparison of results.<br>• Participation fee required.<br>• Assessments occur only several times per year (e.g., quarterly). |
| Comparability testing | In veterinary medicine, a common example is using a reference laboratory to verify results from an in-clinic POCA. This may be done by sending an aliquot of patient sample or QCM to the reference laboratory. Regularly scheduled comparability testing could be used as a form of external QC. | Currently, is more easily available to POCA operators than an appropriate EQA program. | • May compare results from two different analytical methods<br>• Sample stability may influence results |
| Non-statistical QA procedures | • Correlation of laboratory data and clinical signs<br>• Repeat criteria<br>• Medical review criteria<br>• Blood smear review<br>• Monitoring of patient result trends<br>• Documentation of personnel training<br>• Documentation of POCA maintenance<br>• Monitoring (if applicable) of water quality, power source quality, temperature of refrigerators and water baths, calibration of pipettes and centrifuges | • Many procedures are common sense and do not require special training in QA/QC.<br>• Helps overcome limitations inherent in automated instruments (e.g. blood smear evaluation).<br>• May help catch egregious errors in POCA function. | • Guided by written policies and SOPs<br>• Documented in forms, logs, and reports |

| | |
|---|---|
| EQA | External quality assessment |
| QA | quality assurance |
| QC | quality control |
| QCM | quality control materials |
| Ped | probability of analytical error detection (a control rule's "sensitivity" for detecting analytical error) |
| Pfr | probability of false rejection of control results (a control rule's "specificity" for detecting analytical error) |
| SOP | standard operating procedure |

*Table 2 Instrument Performance Specifications Required for Application of the $1_{3s}$ Control Rule, Using TEa Values Recommended by ASVCP*

| **Allowable Total Error (TEa)[5]** | **1-3s rule with n = 1 (Ped ≥ 85% & Pfr = 0%)**<br><br>**Bias(B) and coefficient of variation (CV) required*** | **1-3s rule with n =2 (Ped ≥ 90% & Pfr = 0%)**<br><br>**Bias(B) and coefficient of variation (CV) required*** |
|---|---|---|
| 5% | B ≤ 1.0, with CV ≤ 0.71 | B ≤ 1.0, with CV ≤ 0.77 |
| 5% | B > 1.0 but ≤ 2.0, with CV ≤0.50 | B > 1.0 but ≤ 2.0, with CV ≤ 0.58 |
| 5% | B > 2.0 but ≤ 3.0, with CV ≤0.3 | B > 2.0 but ≤ 3.0 with CV ≤ 0.37 |
| 5% | B >3.0 but ≤4.0, with CV ≤ 0.1 | B > 3.0 but ≤ 4.0 with CV ≤ 0.19 |
| | | |
| 10% | B ≤ 1.0, with CV ≤ 1.55 | B ≤ 1.0, with CV ≤ 1.75 |
| 10% | B > 1.0 but ≤ 2.0, with CV ≤ 1.37 | B > 1.0 but ≤ 2.0, with CV ≤ 1.75 |
| 10% | B > 2.0 but ≤ 3.0. with CV ≤ 1.24 | B > 2.0 but ≤ 3.0. with CV ≤ 1.55 |
| 10% | B > 3.0 but ≤ 4.0, with CV ≤ 1.05 | B > 3.0 but ≤ 4.0, with CV ≤ 1.16 |
| 10% | B > 4.0 but ≤ 5.0, with CV ≤ 0.87 | B > 4.0 but ≤ 5.0, with CV ≤ 0.97 |
| 10% | B > 5.0 but ≤ 6.0, with CV≤ 0.71 | B > 5.0 but ≤ 6.0, with CV≤ 0.77 |
| 10% | B > 6.0 but ≤ 7.0 with CV ≤ 0.53 | B > 6.0 but ≤ 7.0 with CV ≤ 0.58 |
| | | |
| 12% | B ≤ 1.0, with CV ≤ 1.88 | B ≤ 1.0, with CV ≤ 2.13 |
| 12% | B > 1.0 but ≤ 2.0, with CV ≤ 1.68 | B > 1.0 but ≤ 2.0, with CV ≤ 1.94 |
| 12% | B > 2.0 but ≤ 3.0. with CV ≤ 1.55 | B > 2.0 but ≤ 3.0. with CV ≤ 1.75 |
| 12% | B > 3.0 but ≤ 4.0, with CV ≤ 1.35 | B > 3.0 but ≤ 4.0, with CV ≤ 1.55 |
| 12% | B > 4.0 but ≤ 5.0, with CV ≤ 1.24 | B > 4.0 but ≤ 5.0, with CV ≤ 1.36 |
| 12% | B > 5.0 but ≤ 6.0, with CV≤ 1.05 | B > 5.0 but ≤ 6.0, with CV≤ 1.16 |
| 12% | B > 6.0 but ≤ 7.0 with CV ≤ 0.85 | B > 6.0 but ≤ 7.0 with CV ≤ 0.97 |
| 12% | B > 7.0 but ≤ 8.0, with CV ≤ 0.71 | B > 7.0 but ≤ 8.0, with CV ≤ 0.77 |
| 12% | B > 8.0 but ≤ 9.0, with CV ≤ 0.53 | B > 8.0 but ≤ 9.0, with CV ≤ 0.58 |
| | | |
| 15% | B ≤ 1.0, with CV ≤ 2.45 | B ≤ 1.0, with CV ≤ 2.72 |
| 15% | B > 1.0 but ≤ 2.0, with CV ≤ 2.30 | B > 1.0 but ≤ 2.0, with CV ≤ 2.52 |
| 15% | B > 2.0 but ≤ 3.0. with CV ≤ 2.13 | B > 2.0 but ≤ 3.0. with CV ≤ 2.32 |
| 15% | B > 3.0 but ≤ 4.0, with CV ≤ 1.95 | B > 3.0 but ≤ 4.0, with CV ≤ 2.14 |
| 15% | B > 4.0 but ≤ 5.0, with CV ≤ 1.76 | B > 4.0 but ≤ 5.0, with CV ≤ 1.94 |
| 15% | B > 5.0 but ≤ 6.0, with CV≤ 1.58 | B > 5.0 but ≤ 6.0, with CV≤ 1.75 |
| 15% | B > 6.0 but ≤ 7.0 with CV ≤ 1.42 | B > 6.0 but ≤ 7.0 with CV ≤ 1.55 |
| 15% | B > 7.0 but ≤ 8.0, with CV ≤ 1.24 | B > 7.0 but ≤ 8.0, with CV ≤ 1.36 |
| 15% | B > 8.0 but ≤ 9.0, with CV ≤ 1.05 | B > 8.0 but ≤ 9.0, with CV ≤ 1.16 |
| 15% | B > 9.0 but ≤ 10.0, with CV ≤ 0.88 | B > 9.0 but ≤ 10.0, with CV ≤ 0.97 |
| 15% | B > 10.0 but ≤ 11.0, with CV ≤ 0.71 | B > 10.0 but ≤ 11.0, with CV ≤ 0.77 |
| 15% | B > 11.0 but ≤ 12.0, with CV ≤ 0.53 | B > 11.0 but ≤ 12.0, with CV ≤ 0.58 |

| | | |
|---|---|---|
| 20% | B ≤ 1.0, with CV ≤ 3.37 | B ≤ 1.0, with CV ≤ 3.70 |
| 20% | B > 1.0 but ≤ 2.0, with CV ≤ 3.18 | B > 1.0 but ≤ 2.0, with CV ≤ 3.50 |
| 20% | B > 2.0 but ≤ 3.0. with CV ≤ 3.01 | B > 2.0 but ≤ 3.0. with CV ≤ 3.30 |
| 20% | B > 3.0 but ≤ 4.0, with CV ≤ 2.84 | B > 3.0 but ≤ 4.0, with CV ≤ 3.10 |
| 20% | B > 4.0 but ≤ 5.0, with CV ≤ 2.66 | B > 4.0 but ≤ 5.0, with CV ≤ 2.90 |
| 20% | B > 5.0 but ≤ 6.0, with CV≤ 2.48 | B > 5.0 but ≤ 6.0, with CV≤ 2.72 |
| 20% | B > 6.0 but ≤ 7.0 with CV ≤ 2.30 | B > 6.0 but ≤ 7.0 with CV ≤ 2.52 |
| 20% | B > 7.0 but ≤ 8.0, with CV ≤ 2.11 | B > 7.0 but ≤ 8.0, with CV ≤ 2.33 |
| 20% | B > 8.0 but ≤ 9.0, with CV ≤ 1.95 | B > 8.0 but ≤ 9.0, with CV ≤ 2.13 |
| 20% | B > 9.0 but ≤ 10.0, with CV ≤ 1.77 | B > 9.0 but ≤ 10.0, with CV ≤ 1.94 |
| 20% | B > 10.0 but ≤ 11.0, with CV ≤ 1.57 | B > 10.0 but ≤ 11.0, with CV ≤ 1.75 |
| 20% | B > 11.0 but ≤ 12.0, with CV ≤ 1.42 | B > 11.0 but ≤ 12.0, with CV ≤ 1.55 |
| 20% | B > 12.0 but ≤ 13.0, with CV ≤ 1.24 | B > 12.0 but ≤ 13.0, with CV ≤ 1.36 |
| 20% | B > 13.0 but ≤ 14.0, with CV ≤ 1.06 | B > 13.0 but ≤ 14.0, with CV ≤ 1.16 |
| 20% | B > 14.0 but ≤ 15.0, with CV ≤ 0.88 | B > 14.0 but ≤ 15.0, with CV ≤ 0.97 |
| 20% | B > 15.0 but ≤ 16.0, with CV ≤ 0.71 | B > 15.0 but ≤ 16.0, with CV ≤ 0.77 |
| 20% | B > 16.0 but ≤ 17.0, with CV ≤ 0.53 | B > 16.0 but ≤ 17.0, with CV ≤ 0.58 |
| | | |
| 25% | B ≤ 1.0, with CV ≤ 4.26 | B ≤ 1.0, with CV ≤ 4.65 |
| 25% | B > 1.0 but ≤ 2.0, with CV ≤ 4.05 | B > 1.0 but ≤ 2.0, with CV ≤ 4.45 |
| 25% | B > 2.0 but ≤ 3.0. with CV ≤ 3.90 | B > 2.0 but ≤ 3.0. with CV ≤ 4.28 |
| 25% | B > 3.0 but ≤ 4.0, with CV ≤ 3.72 | B > 3.0 but ≤ 4.0, with CV ≤ 4.06 |
| 25% | B > 4.0 but ≤ 5.0, with CV ≤ 3.55 | B > 4.0 but ≤ 5.0, with CV ≤ 3.88 |
| 25% | B > 5.0 but ≤ 6.0, with CV≤ 3.37 | B > 5.0 but ≤ 6.0, with CV≤ 3.70 |
| 25% | B > 6.0 but ≤ 7.0 with CV ≤ 3.19 | B > 6.0 but ≤ 7.0 with CV ≤ 3.50 |
| 25% | B > 7.0 but ≤ 8.0, with CV ≤ 3.01 | B > 7.0 but ≤ 8.0, with CV ≤ 3.29 |
| 25% | B > 8.0 but ≤ 9.0, with CV ≤ 2.84 | B > 8.0 but ≤ 9.0, with CV ≤ 3.10 |
| 25% | B > 9.0 but ≤ 10.0, with CV ≤ 2.66 | B > 9.0 but ≤ 10.0, with CV ≤ 2.90 |
| 25% | B > 10.0 but ≤ 11.0, with CV ≤ 2.48 | B > 10.0 but ≤ 11.0, with CV ≤ 2.72 |
| 25% | B > 11.0 but ≤ 12.0, with CV ≤ 2.30 | B > 11.0 but ≤ 12.0, with CV ≤ 2.55 |
| 25% | B > 12.0 but ≤ 13.0, with CV ≤ 2.13 | B > 12.0 but ≤ 13.0, with CV ≤ 2.32 |
| 25% | B > 13.0 but ≤ 14.0, with CV ≤ 1.95 | B > 13.0 but ≤ 14.0, with CV ≤ 2.13 |
| 25% | B > 14.0 but ≤ 15.0, with CV ≤ 1.77 | B > 14.0 but ≤ 15.0, with CV ≤ 1.94 |
| 25% | B > 15.0 but ≤16.0, with CV ≤ 1.59 | B > 15.0 but ≤16.0, with CV ≤ 1.74 |
| 25% | B > 16.0 but ≤ 17.0, with CV ≤ 1.42 | B > 16.0 but ≤ 17.0, with CV ≤ 1.55 |
| 25% | B > 17.0 but ≤ 18.0, with CV ≤ 1.24 | B > 17.0 but ≤ 18.0, with CV ≤ 1.36 |
| 25% | B > 18.0 but ≤ 19.0, with CV ≤ 1.06 | B > 18.0 but ≤ 19.0, with CV ≤ 1.16 |
| 25% | B > 19.0 but ≤ 20.0, with CV ≤ 0.88 | B > 19.0 but ≤ 20.0, with CV ≤ 0.97 |
| 25% | B > 20.0 but ≤ 21.0, with CV ≤ 0.71 | B > 20.0 but ≤ 21.0, with CV ≤ 0.77 |
| 25% | B > 21.0 but ≤ 22.0, with CV ≤ 0.53 | B > 21.0 but ≤ 22.0, with CV ≤ 0.58 |
| | | |
| 30% | B ≤ 1.0, with CV ≤ 5.14 | B ≤ 1.0, with CV ≤ 5.84 |
| 30% | B > 1.0 but ≤ 2.0, with CV ≤ 4.97 | B > 1.0 but ≤ 2.0, with CV ≤ 5.65 |
| 30% | B > 2.0 but ≤ 3.0. with CV ≤ 4.78 | B > 2.0 but ≤ 3.0. with CV ≤ 5.46 |
| 30% | B > 3.0 but ≤ 4.0, with CV ≤ 4.61 | B > 3.0 but ≤ 4.0, with CV ≤ 5.26 |

| | | |
|---|---|---|
| 30% | B > 4.0 but ≤ 5.0, with CV ≤ 4.43 | B > 4.0 but ≤ 5.0, with CV ≤ 5.05 |
| 30% | B > 5.0 but ≤ 6.0, with CV≤ 4.25 | B > 5.0 but ≤ 6.0, with CV≤ 4.67 |
| 30% | B > 6.0 but ≤ 7.0 with CV ≤ 4.08 | B > 6.0 but ≤ 7.0 with CV ≤ 4.44 |
| 30% | B > 7.0 but ≤ 8.0, with CV ≤ 3.90 | B > 7.0 but ≤ 8.0, with CV ≤ 4.25 |
| 30% | B > 8.0 but ≤ 9.0, with CV ≤ 3.72 | B > 8.0 but ≤ 9.0, with CV ≤ 4.06 |
| 30% | B > 8.0 but ≤ 10.0, with CV ≤ 3.55 | B > 9.0 but ≤ 10.0, with CV ≤ 3.89 |
| 30% | B > 10.0 but ≤ 11.0, with CV ≤ 3.37 | B > 10.0 but ≤ 11.0, with CV ≤ 3.70 |
| 30% | B > 11.0, but ≤ 12.0, with CV ≤ 3.19 | B > 11.0, but ≤ 12.0, with CV ≤ 3.50 |
| 30% | B > 12.0, but ≤ 13.0, with CV ≤ 3.01 | B > 12.0, but ≤ 13.0, with CV ≤ 3.30 |
| 30% | B > 13.0 but ≤ 14.0, with CV ≤ 2.84 | B > 13.0 but ≤ 14.0, with CV ≤ 3.10 |
| 30% | B > 14.0 but ≤ 15.0, with CV ≤ 2.66 | B > 14.0 but ≤ 15.0, with CV ≤ 2.90 |
| 30% | B > 15.0 but ≤ 16.0, with CV ≤ 2.48 | B > 15.0 but ≤ 16.0, with CV ≤ 2.72 |
| 30% | B > 16.0 but ≤ 17.0, with CV ≤ 2.30 | B > 16.0 but ≤ 17.0, with CV ≤ 2.52 |
| 30% | B > 17.0 but ≤ 18.0, with CV ≤ 2.13 | B > 17.0 but ≤ 18.0, with CV ≤ 2.33 |
| 30% | B > 18.0 but ≤19.0, with CV ≤ 1.95 | B > 18.0 but ≤19.0, with CV ≤ 2.13 |
| 30% | B > 19.0 but ≤ 20.0, with CV ≤ 1.77 | B > 19.0 but ≤ 20.0, with CV ≤ 1.94 |
| 30% | B > 20.0 but ≤ 21.0, with CV ≤ 1.59 | B > 20.0 but ≤ 21.0, with CV ≤ 1.75 |
| 30% | B > 21.0 but ≤ 22.0 , with CV ≤ 1.42 | B > 21.0 but ≤ 22.0 , with CV ≤ 1.55 |
| 30% | B > 22.0 but ≤ 23.0, with CV ≤ 1.24 | B > 22.0 but ≤ 23.0, with CV ≤ 1.36 |
| 30% | B > 23.0 but ≤ 24.0, with CV ≤ 1.06 | B > 23.0 but ≤ 24.0, with CV ≤ 1.16 |
| 30% | B > 24.0 but ≤ 25.0, with CV ≤ 0.88 | B > 24.0 but ≤ 25.0, with CV ≤ 0.97 |
| 30% | B > 25.0 but ≤ 26.0, with CV ≤ 0.71 | B > 25.0 but ≤ 26.0, with CV ≤ 0.78 |
| 30% | B > 26.0 but ≤ 27.0, with CV ≤ 0.54 | B > 26.0 but ≤ 27.0, with CV ≤ 0.58 |
| | | |
| 40% | B ≤ 2.0, with CV ≤ 6.74 | B ≤ 2.0, with CV ≤ 7.40 |
| 40% | B > 2.0 but ≤ 4.0, with CV ≤ 6.39 | B > 2.0 but ≤ 4.0, with CV ≤ 6.95 |
| 40% | B > 4.0 but ≤ 6.0. with CV ≤ 6.03 | B > 4.0 but ≤ 6.0. with CV ≤ 6.57 |
| 40% | B > 6.0 but ≤ 8.0, with CV ≤ 5.68 | B > 6.0 but ≤ 8.0, with CV ≤ 6.23 |
| 40% | B > 8.0 but ≤ 10.0, with CV ≤ 5.32 | B > 8.0 but ≤ 10.0, with CV ≤ 5.84 |
| 40% | B > 10.0 but ≤ 12.0, with CV≤ 4.97 | B > 10.0 but ≤ 12.0, with CV≤ 5.45 |
| 40% | B > 12. 0 but ≤ 14.0 with CV ≤ 4.61 | B > 12. 0 but ≤ 14.0 with CV ≤ 5.05 |
| 40% | B > 14.0 but≤ 16.0, with CV ≤ 4.26 | B > 14.0 but≤ 16.0, with CV ≤ 4.66 |
| 40% | B > 16.0 but ≤ 18.0, with CV ≤ 3.90 | B > 16.0 but ≤ 18.0, with CV ≤ 4.28 |
| 40% | B > 18.0 but ≤ 20.0, with CV ≤ 3.55 | B > 18.0 but ≤ 20.0, with CV ≤ 3.88 |
| 40% | B > 20.0 but ≤ 22.0, with CV ≤ 3.19 | B > 20.0 but ≤ 22.0, with CV ≤ 3.50 |
| 40% | B > 22.0 but ≤ 24.0, with CV ≤ 2.84 | B > 22.0 but ≤ 24.0, with CV ≤ 3.09 |
| 40% | B > 24.0 but ≤ 26.0, with CV ≤ 2.48 | B > 24.0 but ≤ 26.0, with CV ≤ 2.72 |
| 40% | B > 26.0 but ≤ 28.0, with CV ≤ 2.13 | B > 26.0 but ≤ 28.0, with CV ≤ 2.33 |
| 40% | B > 28.0 but ≤ 30.0, with CV ≤ 1.77 | B > 28.0 but ≤ 30.0, with CV ≤ 1.94 |
| 40% | B > 30.0 but ≤ 32.0, with CV ≤ 1.42 | B > 30.0 but ≤ 32.0, with CV ≤ 1.55 |
| 40% | B > 32.0 but ≤ 34.0, with CV ≤ 1.06 | B > 32.0 but ≤ 34.0, with CV ≤ 1.16 |
| 40% | B > 34.0 but ≤ 36.0,with CV ≤ 0.68 | B > 34.0 but ≤ 36.0,with CV ≤ 0.77 |
| 40% | B > 36.0 but ≤38.0, with CV ≤ 0.35 | B > 36.0 but ≤38.0, with CV ≤ 0.38 |
| | | |

| | | |
|---|---|---|
| 70% | B ≤ 2.0, with CV ≤ 12.00 | B ≤ 2.0, with CV ≤ 13.70 |
| 70% | B > 2.0 but ≤ 4.0, with CV ≤ 11.72 | B > 2.0 but ≤ 4.0, with CV ≤ 13.35 |
| 70% | B > 4.0 but ≤ 6.0. with CV ≤ 11.35 | B > 4.0 but ≤ 6.0. with CV ≤ 12.95 |
| 70% | B > 6.0 but ≤ 8.0, with CV ≤ 11.00 | B > 6.0 but ≤ 8.0, with CV ≤ 12.54 |
| 70% | B > 8.0 but ≤ 10.0, with CV ≤ 10.65 | B > 8.0 but ≤ 10.0, with CV ≤ 12.12 |
| 70% | B > 10.0 but ≤ 12.0, with CV≤ 10.27 | B > 10.0 but ≤ 12.0, with CV≤ 11.72 |
| 70% | B > 12.0 but ≤ 14.0 with CV ≤ 9.92 | B > 12.0 but ≤ 14.0 with CV ≤ 11.27 |
| 70% | B > 14.0 but ≤ 16.0, with CV ≤ 9.58 | B > 14.0 but ≤ 16.0, with CV ≤ 10.92 |
| 70% | B > 16.0 but ≤ 18.0, with CV ≤ 9.23 | B > 16.0 but ≤ 18.0, with CV ≤ 10.52 |
| 70% | B > 18.0 but ≤ 20.0, with CV ≤ 8.85 | B > 18.0 but ≤ 20.0, with CV ≤ 10.11 |
| 70% | B > 20.0 but ≤ 22.0, with CV ≤ 8.52 | B > 20.0 but ≤ 22.0, with CV ≤ 9.65 |
| 70% | B > 22.0 but ≤ 24.0, with CV ≤ 8.16 | B > 22.0 but ≤ 24.0, with CV ≤ 9.30 |
| 70% | B > 24.0 but ≤ 26.0, with CV ≤ 7.81 | B > 24.0 but ≤ 26.0, with CV ≤ 8.90 |
| 70% | B > 26.0 but ≤ 28.0, with CV ≤ 7.45 | B > 26.0 but ≤ 28.0, with CV ≤ 8.45 |
| 70% | B > 28.0 but ≤ 30.0 with CV ≤ 7.10 | B > 28.0 but ≤ 30.0 with CV ≤ 8.45 |
| 70% | B > 30.0 but ≤ 32.0, with CV ≤ 6.74 | B > 30.0 but ≤ 32.0, with CV ≤ 8.08 |
| 70% | B > 32.0 but ≤ 34.0, with CV ≤ 6.38 | B > 32.0 but ≤ 34.0, with CV ≤ 7.64 |
| 70% | B > 34.0 but ≤ 36.0, with CV ≤ 6.02 | B > 34.0 but ≤ 36.0, with CV ≤ 7.28 |
| 70% | B > 36.0 but ≤ 38.0, with CV ≤ 5.68 | B > 36.0 but ≤ 38.0, with CV ≤ 6.84 |
| 70% | B > 38.0 but ≤ 40.0, with CV ≤ 5.32 | B > 38.0 but ≤ 40.0, with CV ≤ 6.45 |
| 70% | B > 40.0 but ≤ 42.0, with CV ≤ 4.97 | B > 40.0 but ≤ 42.0, with CV ≤ 6.07 |
| 70% | B > 42.0 but ≤ 44.0 with CV ≤ 4.61 | B > 42.0 but ≤ 44.0 with CV ≤ 5.25 |
| 70% | B > 44.0 but ≤ 46.0, with CV ≤ 4.26 | B > 44.0 but ≤ 46.0, with CV ≤ 4.83 |
| 70% | B > 46.0 but ≤ 48.0, with CV ≤ 3.90 | B > 46.0 but ≤ 48.0, with CV ≤ 4.45 |
| 70% | B > 48.0 but ≤ 50.0, with CV ≤ 3.55 | B > 48.0 but ≤ 50.0, with CV ≤ 4.04 |
| 70% | B > 50.0 but ≤ 52.0, with CV ≤ 3.19 | B > 50.0 but ≤ 52.0, with CV ≤ 3.64 |
| 70% | B > 52.0 but ≤ 54.0, with CV ≤ 2.84 | B > 52.0 but ≤ 54.0, with CV ≤ 3.22 |
| 70% | B > 54.0 but ≤ 56.0, with CV ≤ 2.48 | B > 54.0 but ≤ 56.0, with CV ≤ 2.83 |
| 70% | B > 56.0 but ≤ 58.0, with CV ≤ 2.13 | B > 56.0 but ≤ 58.0, with CV ≤ 2.42 |
| 70% | B > 58.0 but ≤ 60.0, with CV ≤ 1.77 | B > 58.0 but ≤ 60.0, with CV ≤ 2.02 |
| 70% | B > 60.0 but ≤ 62.0, with CV ≤ 1.42 | B > 60.0 but ≤ 62.0, with CV ≤ 1.61 |
| 70% | B > 62.0 but ≤ 64.0, with CV ≤ 1.06 | B > 62.0 but ≤ 64.0, with CV ≤ 1.21 |
| 70% | B > 64.0 but ≤ 66.0, with CV ≤ 0.71 | B > 64.0 but ≤ 66.0, with CV ≤ 0.80 |
| 70% | B > 66.0 but ≤ 68.0, with CV ≤ 0.35 | B > 66.0 but ≤ 68.0, with CV ≤ 0.40 |

* - CV carried out to ≤ 0.6.

These recommendations are extrapolated from Westgard's EZRules3 QC validation software[29]; they represent a simplified approach and do not cover all possible combinations of bias and CV.

TEa Allowable Total Error (%)

Ped Probability of analytical error detection (a control rule's "sensitivity" for detecting analytical error)

Pfr Probability of false rejection of control results (a control rule's "specificity" for detecting analytical error)

B% Absolute Bias (%)

CV% Coefficient of Variation (%)

## 2.13 Resources

### *2.13.1 Journal Articles*


- Vap LM, Harr KE, Arnold JE, Freeman KP, Getzy K, Lester S, Friedrichs KR. ASVCP quality assurance guidelines: control of preanalytical and analytical factors for hematology for mammalian and nonmammalian species, hemostasis, and crossmatching in veterinary laboratories. *Veterinary Clinical Pathology* 2012; 41:8-17.
- Gunn-Christie RG, Flatland B, Friedrichs KR, Szladovits B, Harr KE, Ruotsalo K, Knoll JS, Wamsley HL, Freeman KP. ASVCP quality assurance guidelines: control of preanalytical, analytical, and postanalytical factors for urinalysis, cytology, and clinical chemistry in veterinary laboratories. *Veterinary Clinical Pathology* 2012; 41:18-26.
- Flatland B, Freeman KP, Friedrichs KR, Vap LM, Getzy KM, Evans EW, Harr KE. ASVCP quality assurance guidelines: control of general analytical factors in veterinary laboratories. *Veterinary Clinical Pathology* 2010; 39:264-277.
- Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhardt KF, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology* 2012; 41:441-453.
- Geffré A, Friedrichs K, Harr K, Concordet D, Trumel C, Braun J. Reference values: a review. *Veterinary Clinical Pathology*. 2009; 38:288-298.

### *2.13.2 Books*


- Bellamy JEC, Olexson DW. *Quality Assurance Handbook for Veterinary Laboratories*. Ames, IA: Iowa State University Press; 2000.
- Westgard JO. *Basic Planning for Quality*. Madison, WI: Westgard QC, Inc.; 2000.
- Westgard JO. *Basic Method Validation*. 3rd ed. Madison, WI: Westgard QC, Inc.; 2008.
- Westgard JO. *Basic QC Practices*. 3rd ed. Madison, WI: Westgard QC, Inc.; 2010.


### *2.13.3 Web-based resources*

| **Resource** | **URL** | **Potential Uses** |
|---|---|---|
| American Society for Veterinary Clinical Pathology | www.asvcp.org | Guideline documents freely available at http://www.asvcp.org/pubs/qas/index.cfm |
| Veterinary Information Network | www.vin.com | Offers continuing education courses and rounds. |
| Veterinary Information Network QC Calculator | http://www.vin.com/doc/?id=5200884 | A "QC calculator" that can be used to assist QC validation. |
| Westgard QC | www.westgard.com | Offers information about and tools for QA/QC, including essays, lessons, calculators, books, software, and continuing education events. |

### *2.13.4 Computer software programs*

| Name | Manufacturer | URL | Software Type | Potential Uses |
|---|---|---|---|---|
| Excel | Microsoft Corporation (Redmond, WA, USA) | www.microsoft.com | Data spreadsheet | Organizing and archiving data, performing calculations and basic statistics |
| MedCalc | MedCalc (Mariakerke, Belgium) | www.medcalc.org | Medical statistical software | Organizing and archiving data, performing statistical analysis, creating control charts |
| EZRules3 | Westgard QC (Madison, WI, USA) | http://www.westgard.com/software.htm | QC validation software | QC validation |

## 2.14 Example forms and logs.

Please see separate Appendix.

# Section 3 – Recommendations for Point-of-Care Hematology Testing in Domestic Mammals

Flatland B, Vap LM

**Acknowledgement**

The authors thank the faculty and staff of the Clinical Pathology Laboratory at the University of Tennessee for providing blood smear and medical review criteria. The authors thank Dr. Joanne Messick for her review of this material during adaptation of a draft version of these guidelines for a chapter in *Veterinary Clinics of North America*.[1]

## Contents

### 3.1 Abbreviations and definitions

Definitions and formulas are given for selected hematology terms only. For definitions and formulas for other terms, please refer to the glossary in Section 2 *Quality Assurance Guidelines for Veterinary Point-of-Care Chemistry Testing.*

| | |
|---|---|
| ASVCP | American Society for Veterinary Clinical Pathology |
| CBC | Complete blood count. The combined reporting of a patient's erythrogram, leukogram, and thrombogram. |
| CLSI | Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards, NCCLS) |
| CV | Coefficient of variation |
| EDTA | Ethylenediaminetetraacetic acid. Anticoagulant used for hematology testing in mammals. |
| Hct | Hematocrit. Percentage of RBCs in blood as calculated by automated instrument using RBC concentration and MCV). |
| Hgb | Hemoglobin concentration |
| MCHC | Mean cell (corpuscular) hemoglobin concentration. The formula for MCHC[2] is $MCHC = \frac{[Hgb] \times 100}{Hct}$. |
| MCV | Mean cell (corpuscular) volume. MCV may be directly measured by some instruments. The formula for MCV[2] is $MCV = \frac{Hct \times 10}{[RBC]}$. |

| | |
|---|---|
| Neut | Neutrophil |
| PCV | Packed cell volume. Percentage of RBCs in blood as determined by centrifugation of a microhematocrit tube and measured using a capillary card reader (a.k.a. “spun hematocrit”). |
| Plt | Platelet |
| POCT | Point-of-care test(ing) |
| POCA | Point-of-care analyzer(s) or analysis |
| QA | Quality assurance |
| QALS | Quality Assurance and Laboratory Standards Committee (of the ASVCP) |
| QC | Quality control. See section 2 for definitions of internal QC, external QC, and in-clinic QC. |
| QCM | Quality control material |
| RBC | Red blood cell (erythrocyte) |
| TEa | Allowable total error (a quality requirement) |
| $TE_{obs}$ | Observed (calculated) total error |
| TNCC | Total nucleated cell count. Absolute count of all nucleated cells as counted by an automated hematology analyzer (includes leukocytes and nucleated erythroid cells). |
| WBC | White blood cell (leukocyte) |

### 3.2 Hematology point-of-care analyzer selection

Hematology instruments use three methods for counting, sizing, and differentiating cells: centrifugation, impedance counting, and laser light scatter (flow cytometric).[3] The centrifugal method is an older, less complex technology but still available. Impedance-based analyzers are widely available, while light scatter is relatively new to POCA in veterinary medicine. Analyzers using laser light-scatter may also incorporate impedance-based methods. A detailed review of these methods can be found in other resources.[3-5] Factors that may influence selection of a particular hematology instrument for a given veterinary hospital include:

- Direct and indirect costs
    - Instrument purchase vs. lease
    - Reagents
        - Closed and open container shelf-lives (is shelf-life practical given anticipated usage?)
    - Presence of absence of internal instrument QC functions
    - Quality control materials (QCM) used for external QC
        - Availability
        - Cost
        - Closed and open container shelf-lives
    - Availability and cost of external quality assurance (EQA) programs (proficiency testing)
    - Cost and length of maintenance/service contracts

    - Time investments
        - Operator training and continuing education
        - Instrument maintenance and trouble-shooting
        - Internal and external QC
        - Turnaround time/throughput capability
        - Maintaining inventory
- Distinguishing test menu items
    - How extensive is the WBC differential (i.e., 3-part or 5-part)?
    - Fibrinogen estimate, microfilaria detection (centrifugal only)
    - Erythrocyte indices (MCV and MCHC by impedance or light scatter)
    - Mean platelet volume (MPV by impedance or light scatter)
    - Reticulocyte count (measured by light scatter, estimated by centrifugal)
- Species capabilities/validated for species of interest
- Sample
    - Volume requirements
    - Sample type capabilities (some POCA can also assay cavity fluids and urine)
- Instrument
    - Size (“footprint”)
    - Ease of instrument and software operation
    - Ambulatory capability, if needed
    - Environmental requirements (temperature, humidity, vibration)
    - Data management

        - Report format
        - Data storage and recall
        - Flags: operational, abnormal values, etc.
        - Histograms or cytograms for interpretation
        - Presentation and archiving of control data (e.g., can the instrument create control charts?)
    - Maintenance requirements
    - Analytical performance (minimum performance specifications may be available from the instrument manufacturer)
- Health and safety considerations
    - Waste generated: amount, type, and packaging/disposal required
    - Infection control
- Customer support
    - Training and continuing education for instrument operators
    - Technical service provided by the manufacturer
- Instrument reputation
    - Feedback from other instrument users
    - Analytical performance data reported in medical literature

## 3.3 Unique aspects of hematology testing

### *3.3.1. Mammals vs. non-mammals*

In general, enumeration of hemic cells from birds, reptiles, amphibians, and fish is not supported by manufacturers of automated hematology analyzers due to the presence of nucleated RBCs and thrombocytes in these species that interfere with instrument counting functions.[6] Hemocytometers and specialized pipette systems are used for exotic animal hematology and are not covered by these guidelines. More information about exotic animal hematology can be found in other resources.[7-9] Recommendations presented herein specifically refer to hematology testing of mammalian blood samples.

### *3.3.2 The importance of blood smear review*

In addition to numerical results (cell counts and indices) reported by automated hematology analyzers, evaluation of blood cell morphology is a critical aspect of hematology testing. Certain morphologic changes (e.g., presence of RBC poikilocytosis, Heinz bodies, neutrophil toxic change, hemoparasites and other infectious organisms) simply cannot be detected by automated analyzers (of any type), and evaluation of a well-made blood smear is an essential quality assurance (QA) procedure in hematology testing that may additionally be diagnostic. Other morphologic changes in hemic cells may either go undetected by automated instruments or trigger instrument flags or abnormalities in histograms or cytograms, and blood smear review is required to clarify and further elucidate the abnormality (e.g., presence of nucleated RBCs, neutrophil left shift, basophilia, neoplastic cells, and clumped, large, or misshapen platelets). Blood smear preparation and evaluation technique influences

interpretation and personnel must have proper training. Instruction is available in published literature, and interested readers are referred to other resources.[10, 11] In addition to smear preparation technique, microscope maintenance and technique are key components of successful blood smear review. Stain should be handled according to manufacturer's instructions and changed regularly to keep it fresh and free of microorganisms.

Ideally, blood smears should be reviewed for all CBCs performed by the clinic. At minimum, blood smears should be reviewed for CBCs from clinically ill patients and CBCs yielding unexpected or suspicious results. A qualified individual such as a veterinarian or veterinary technician should assess the respective densities and morphology of RBCs, WBCs, and platelets to look for platelet clumping, and compare their subjective impression of the blood smear to numerical data as well as any instrument flags, histograms, or cytograms from the analyzer. Unexpected or suspicious instrument data indicate more critical smear review, further evaluation of the patient by the veterinarian, evaluation of the instrument by manufacturer's technical support, and/or referral of the sample to a clinical pathology laboratory. Criteria should be in place to guide use of manual WBC differential counts in place of the automated differential count (**Table 1)**, and medical review criteria should be used (**Table 2**). If nRBC are not included in the 100-cell manual nucleated differential count but are rather counted additionally, and if > 5 nRBCs/100 WBCs are identified, the automated total WBC concentration should be corrected[3], and the absolute leukocyte concentrations should be recalculated using the corrected total WBC count. If nRBC are included in the differential count, corrected WBC counts should be calculated, but absolute leukocyte concentrations do not need to be recalculated.

*3.3.3 Comparability testing in veterinary hematology*

Periodic (quarterly) external quality assessment of hematology instrumentation is recommended to confirm instrument function, reagent stability, and comparability to a reference laboratory. If sending an aliquot of patient sample to a reference laboratory to compare results from an in-house hematology POCA, this should be done within 24 hours of sample acquisition. The blood sample should be refrigerated (4°C) immediately following in-clinic analysis until arrival at the reference laboratory.[12] See section 2 and the ASVCP TEa guideline[13] for additional recommendations regarding comparability testing.

Air-dried blood smears are stable specimens and may last for years if protected from light and moisture (particularly if a coverslip is applied using an appropriate adhesive). Smears that are shipped to reference laboratories for review should be protected from condensation, freezing, and formalin fumes.[7]

**3.4 Minimizing pre-analytical error in hematology testing**

Sample age, collection technique, and handling are critical to production of quality hematology results. Use of written SOPs that describe sample acquisition, handling, and storage is recommended.

*3.4.1 Proper sample handling*

1. Proper sample tube and tube filling. Blood for CBCs should be collected into EDTA (lavender-top) tubes.[12] Tubes are available containing EDTA in liquid ($K_2$-EDTA) or spray-dried ($K_3$-EDTA) forms. $K_2$-EDTA is recommended[14]. EDTA liquid inside blood collection

tubes can dilute samples 1-2% or more, depending on fill-volume. Under-filling these tubes can significantly decrease PCV as a result of RBC shrinkage in the presence of the highly osmolar EDTA. In addition, excess EDTA can falsely increase plasma protein as determined by refractometry, especially in samples from patients with low plasma protein concentration. Filling tubes at least half full is recommended, as this is unlikely to alter clinical interpretation. Small volume tubes are available and should be used for smaller or sick animals.

2. Proper sample mixing. Mixing the collection tube by gentle inversion 8-10 times immediately after filling is recommended to avoid clotting of the sample. When samples rest in a test tube rack, erythrocytes settle to the bottom of the sample tube, leaving nucleated cells and platelets concentrated at the top. Sufficient sample mixing is therefore also crucial immediately prior to sample analysis. The net effect of settling in improperly mixed samples will vary with the location of the instrument needle aspirating the aliquot to be measured.[10]
3. Rejection of clotted samples. Samples containing grossly visible clots should be rejected. The effect of clotting on cell concentrations will be proportional to the clot size and/or number but cannot be accurately predicted by visual inspection of the clot. Small clots may not be grossly visible but can be detected by gently stirring the sample prior to analysis with one or more clean, wooden applicator sticks; clots will adhere to the stick. In addition to affecting cell counts because they trap cells, clots may cause mechanical problems; aspiration of small clots may plug tubing in the analyzer, altering

the accuracy of results for the current or subsequent sample(s).[2] If sample clotting is observed, the sample should be rejected and a new sample obtained.

4. Timely preparation and proper staining of well-made blood smears. Blood smears should be prepared by appropriately trained personnel as soon as possible after collection and stored at room temperature. Only smears of good to excellent quality (having a smooth, uniform, feathered edge, with no holes or gaps in the film of blood) should be examined. Blood smears must be kept away from moisture and formalin fumes. Smears should be stained with a Romanowsky stain that is fresh and uncontaminated by debris or microorganisms.[3]

*3.4.2 Sample characteristics that may adversely affect quality of CBC data*

Certain sample characteristics may adversely affect the quality of CBC data and are reasons for sample rejection or annotation of CBC results:

1. Platelet activation, resulting in clumping, may occur during venipuncture and sample handling and may cause falsely decreased automated platelet counts.[15] Unexpectedly low platelet counts (based on patient condition and other clinical information) should prompt evaluation of a blood smear to look for obvious platelet clumping. The authors have observed that presence, number, and size of platelet clumps are not always reproducible between smears made from the same blood sample. If large clumps are observed on smear review and/or a low automated platelet count (regardless of the degree of observed clumping) requires confirmation, a fresh blood sample should be drawn (using atraumatic venipuncture and conscientious sample handling) and

analyzed. Samples from animals having increased numbers of large platelets in circulation may also yield falsely low automated platelet counts, because analyzers using impedance methods may not be able to distinguish large platelets from RBCs.[16]

2. Agglutination of RBCs may falsely lower RBC concentration and falsely increase MCV[2]; its effect on Hct and MCHC is variable. If severe, agglutination may be detected by examining the sample collection tube walls grossly for clumping. Significant rouleaux can mimic agglutination; distinguishing between these can usually be accomplished with a saline dispersion test. Blood is added to saline on a glass slide to achieve approximately a 1:4 (or greater) dilution and examined under the microscope.[2] Rouleaux should dissipate while agglutination remains.
3. Marked numbers of Heinz bodies or nucleated cells may render the sample turbid, falsely increasing Hgb concentration and subsequently MCHC and MCH, particularly in anemic patients.[17] Heinz bodies may be detected on routine blood smear review and can be confirmed using new methylene blue staining.[2] Presence of Heinz bodies should be noted semiquantitatively (e.g., mild, moderate or marked) or reported as the percentage of RBCs affected on the CBC report.

## 3.5 Minimizing analytical error in hematology testing

### *3.5.1 Frequency of external quality control in hematology testing*

Blood smear review (section 4.2.2) is an important aspect of external QC in hematology testing. Hematology instruments do not use unit devices and may or may not have internal QC

features, depending upon the instrument. Daily external QC consisting of measuring at least one level of assayed QCM is recommended for impedance and light scatter-based instruments, in addition to daily monitoring of any internal QC functions the instrument may possess.

*3.5.2 Statistical QC and use of the $1_{3s}$ control rule in point-of-care hematology testing*

A recent veterinary publication showed that it is possible to perform statistical QC for biochemistry analytes measured using POCA with a simple control rule ($1_{3s}$) and one or two control materials (N = 1 or N = 2)[18]. This control rule can also be considered for statistical QC of hematology POCA, although more study is needed. If statistical QC is to be attempted, sections 2.6 and 2.7 of these guidelines, Rishniw, et al.[18], and the ASVCP TEa guideline[13] may be used as resources.

*3.5.3 Non-statistical QA procedures specific to hematology testing*

1. Documentation of regular maintenance (and regular verification of proper function, if applicable) of microscope, Romanowsky stain, refractometer, and microhematocrit centrifuge is recommended.
2. Correlation of Hct and PCV. Performing a PCV and plasma protein measurement with each CBC is recommended. A spun Hct tube can be used to evaluate plasma characteristics (e.g., look for lipemia, hemolysis, or icterus) and confirm the automated Hct. Because a small amount of plasma and some platelets and leukocytes become trapped within the RBC column inside a microhematocrit tube, PCV may be slightly

higher than Hct as reported by the POCA.[2]. If concurrent PCV and Hct values disagree by greater than 3 L/L, results from both methods should be investigated for potential error.

3. Correlation of Hgb, Hct, and MCHC. Assuming MCHC is within the reference interval, the numerical value of the measured blood hemoglobin (Hgb) concentration should be approximately one-third of the Hct numerical value[2]; if MCHC is abnormal, this relationship may not hold true. If MCHC is within the reference interval (or close to it) and Hgb does not approximately equal one third of the Hct numerical value, then both Hgb and Hct results should be investigated for sources of error. Increased MCHC is almost always an artifact and should prompt investigation for hemolysis, lipemia, or Heinz bodies.[2] Hemolysis falsely decreases the RBC concentration and Hct/PCV. Excessive lipemia may falsely increase Hgb concentration. Either situation results in a falsely increased MCHC and MCH. Plasma should be evaluated for lipemia and hemolysis by inspecting the microhematocrit tube that is centrifuged for a concurrent PCV. The level of lipemia or hemolysis can be reported in a semi-quantitative fashion as slight, moderate, marked or 1+, 2+, 3+. If evidence for these artifacts is lacking, appropriate instrument operation should be confirmed by measuring QCM and technical services should be contacted as needed for assistance.
4. Microscopic evaluation of a well-made blood smear. See section 4.2.2, above.
5. Use of medical review criteria. Use of medical review criteria (**Table 2**) is not unique to hematology testing, but is particularly important in hematology testing in view of the need to correlate morphologic changes with automated instrument data. See section 4.2.2, above.

### 3.6 Minimizing post-analytical errors in hematology testing:

CBC results known to be inaccurate should not be reported.[7] Any observed sample clotting (or other problem with sample quality) should be recorded on the laboratory data report and included in the patient medical record. Automated platelet counts should not be reported if significant platelet clumping is observed on blood smear review; rather, platelets should simply be reported as "clumped"; a semiquantitative estimate such as "clumped, appear adequate" (or increased or decreased, as appropriate) may be added.

Results of the blood smear review should be recorded on or near instrument print-outs of numerical data or on a CBC form; such annotations should be initialed and dated. Whether an automated or manual differential WBC count is used for patient management should be clearly indicated. If computerized medical records are used, CBC data, annotations, and comments should be added into the hospital information system for each patient.

## 3.7 References


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## 3.8 Tables

*Table 1 -- Criteria for Performing an In-Clinic Manual WBC Differential Count by Trained Personnel*

| Presence of . . . | Suggested Cut-Off Value | Comments |
|---|---|---|
| Nucleated RBCs | If more than rare nRBC | Perform and report a manual differential count. Correct automated total WBC for the number of nRBC. If nRBC are not included in the 100-cell manual differential, corrected WBC are calculated when > 5 nRBC are observed and absolute differential counts must be recalculated using the corrected WBC. The latter does not need to be done when nRBC are included in the 100-cell differential count. |
| Neutrophil left shift | > 1 band and/or earlier neutrophils forms (e.g., metamyelocyte) observed | Perform and report a manual differential count, enumerating neutrophil forms (segmented, band, metamyelocyte, etc.) separately. Note whether toxic change is present. Correct absolute differential results (e.g. the neutrophils will now be split into bands and segmented neutrophils ± less mature forms [if present]). |
| Unclassified (unidentified) cells | Any | Perform and report a manual differential count, enumerating the unclassified cells in an "other" category. Describe morphology of the unclassified cells. Recalculate absolute differential results. |
| Subjective impression that automated WBC differential count may not be accurate | N/A | If for any reason the automated WBC differential count is suspect, perform a manual WBC differential count to verify it. E.g., is there is lack of clear distinction between cell types on histograms or cytograms. |

*Table 2 – Suggested Criteria for Medical Review of Blood Smears and Concurrent CBC Data*
Send blood smears and EDTA-anticoagulated whole blood to a board-certified clinical pathologist as needed to confirm abnormal findings.

| Blood Smear | Criteria Triggering a Review |
|---|---|
| Background | Unusual background matrix<br>Unusual background color<br>Organisms or suspected organisms |
| Red Blood Cells | Moderate to marked poikilocytosis of any kind; moderate to severe anemias<br>Reticulocytosis<br>Any Heinz bodies in a non-feline species; > 10% Heinz bodies in cats<br>Any non-routine* inclusions (including organisms or suspected organisms)<br>Basophilic stippling, siderocytes, or Howell-Jolly bodies in dog<br>5 nRBC/100 WBC (or > 10% nRBC if included in the differential count) in non-equine species; any nRBC in horses<br>Abnormal MCV |
| White Blood Cells | Left shift in which bands are ≥ 3% of observed leukocytes, or any left shift in which neutrophil precursors less mature than bands are observed;<br>Leukopenia < 3,000 WBC/μL<br>Any left shift where immature neutrophil forms outnumber segmented neutrophils<br>Leukocytosis > 30,000 WBC/μL in non-ruminants; leukocytosis > 15,000 WBC/μL in ruminants and horses<br>Lymphocytosis > 10,000 cells/μL; Monocytosis > 2,000 cells/μL; Eosinophilia > 2,000 cells/μL; Basophilia > 1,000 cells/μL<br>Any unclassified cells<br>Any organisms or suspected organisms<br>Presence of vacuoles in non-monocytes and abnormal granulation in any leukocyte, other than toxic granulation in neutrophils) |
| Platelets | Platelet count > 900,000 cells/μL (except pigs and ruminants); moderate to severe thrombocytopenia < 100,000 cells/μL<br>Abnormal MPV (if reported by instrument)<br>Suspected inclusions or abnormal granulation |

* Low numbers of Howell-Jolly bodies are occasionally found in blood from healthy cats and horses, but not dogs.

**Acknowledgements**

The Quality Assurance and Laboratory Standards (QALS) Committee thanks the ASVCP membership for their review of and input regarding these guidelines. Additionally, we thank the following invited reviewers for their input (listed alphabetically): Keith DeJong (Abaxis North American Health), Sharon Ehrmeyer (School of Medicine and Public Health, University of Wisconsin-Madison), Debbie Gadomski (National Field Operations Manager, American Animal Hospital Association), Glenn Pettifer (College of Veterinarians in Ontario), Janice Trumpeter (Deputy Executive Director, American Animal Hospital Association), and Glade Weiser (College of Veterinary Medicine and Biomedical Sciences, Colorado State University).

**Appendix – Example Forms and Logs**
**Freeman KP**

For each form or log, a filled-out example is provided first, followed by a blank version. Blank versions may be copied and used.

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**EXAMPLE TEMPERATURE CHART**

Freezer/Refrigerator (specify): Reagent Refrigerator #1

Acceptable Temperature Range: 4 to 8 °C

Frequency of Monitoring: Daily (Monday to Saturday)

Usual setting: 3 (1 warmest to 5 coldest)

| Date | Temperature (°C) | Action(s) Needed or Taken | | Comments | Initials |
|---|---|---|---|---|---|
| | | No Action | Action Taken | | |
| 01-03-09 | 5 | X | | | LAM |
| 03-03-08 | 6 | X | | | KPF |
| 04-03-08 | 10 | | Adjusted thermostat to 2 | | KPF |
| 05-03-09 | 7 | X | | Adjustment appears adequate | LAM |
| 06-03-09 | 8 | X | | | LAM |
| 07-03-09 | 10 | | Defrosted refrigerator | | KPF |
| 08-03-09 | 7 | X | | Defrost appears to have worked | LAM |
| | | | | | |

**BLANK TEMPERATURE CHART**

Freezer/Refrigerator (specify): _____

Acceptable Temperature Range: _____

Frequency of Monitoring: _____

Usual setting: _____

| Date | Temperature (°C) | Action(s) Needed or Taken | | Comments | Initials |
|---|---|---|---|---|---|
| | | No Action | Action Taken | | |
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**EXAMPLE INSTRUMENT CALIBRATION AND SOFTWARE UPDATE LOG**

| Date | Instrument | Update | Done By | Comments |
|---|---|---|---|---|
| 02-03-08 | Animed Chemistry Analyzer | Calibration Disk Installation | LAM | Regular update sent by Animed |
| 05-03-08 | Apple Electrolyte Analyzer | Monthly Calibration | LAM | According to SOP Chem 1.1. Apple Electrolyte Analyzer Monthly Calibration |
| | | | | |

**BLANK INSTRUMENT CALIBRATION AND SOFTWARE UPDATE LOG**

| Date | Instrument | Update | Done By | Comments |
|---|---|---|---|---|
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**EXAMPLE COMPARABILITY TESTING LOG**

| Date | Patient ID | Result needing verification | Send Out | | Agreement Acceptable? | | Investigation, If Not Acceptable | Comments /Initials |
|---|---|---|---|---|---|---|---|---|
| | | | Lab & Date | Result | Yes | No | | |
| 02-02-08 | "Spencer" Jones | Elevated $Ca^{++}$ = 2.7 mmol/L | Idexx, 02-02-08 | 2.9 mmol/L | X | | | Good agreement/KPF |
| 05-03-08 | "Ben" Edwards | Increased PCV = 0.76 L/L | Idexx, 05-03-08 | 0.50 L/L | | X | Discussed QBC result with Customer Services – considered poor sample separation likely. | Monitor 2 additional specimens for agreement of hematocrit/LAM |
| 07-03-08 | "Bow" Freeman | Normal PCV = 0.45 L/L | Idexx, 07-03/08 | 0.46 L/L | X | | | Previous nonagreement specimen ('Ben' Edwards) – checking to see that QBC is OK/LAM |
| | | | | | | | | |

**BLANK COMPARABILITY TESTING LOG**

| Date | Patient ID | Result needing verification | Send Out | | Agreement Acceptable? | | Investigation, If Not Acceptable | Comments /Initials |
|---|---|---|---|---|---|---|---|---|
| | | | Lab & Date | Result | Yes | No | | |
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**EXAMPLE EQUIPMENT PERFORMANCE PROBLEM LOG**

**Instrument Identification:** Apple Electrolyte Analyzer, Serial number 27484

| Date | Problem | Analysis | Action | Comments | Initials |
|---|---|---|---|---|---|
| 01-02-08 | QC Rejection, QC materials 1 and 2 , Test: CK | Reagent out of date | Order new reagent | No CK reported until new reagents available. Anticipated arrival on 04-02-08 | KPF |
| 04-03-08 | Scheduled lamp test shows failure at wavelength 110 | Lamp needs replacing | Lamp replaced | Test of new lamp shows acceptable performance | LAM |
| 22-03-08 | Repeat test of low calcium comes in as within normal limits | Likely random error. No other calciums that day (10 total) were low. All others WNL. Calcium control materials 1 and 2 OK | No action needed. | Sent serum from this dog ('Dotty' Spencer) to reference laboratory for calcium testing – confirms within reference interval. | LAM |
| | | | | | |

**BLANK EQUIPMENT PERFORMANCE PROBLEM LOG**

**Instrument Identification:** _____

| Date | Problem | Analysis | Action | Comments | Initials |
|---|---|---|---|---|---|
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**EXAMPLE LEVEY-JENNINGS CONTROL CHART**

**Instrument Identification:** Animed Chemisry Analyzer, Serial number 28893

**Control Material Name :** DCL Chemistry Control Material Number 1

**Control Material Batch Number:** 90478-B

**Control Material Expiration Date:** 11/2009

**Analyte:** WBC

| | Value | | | | | | |
|---|---|---|---|---|---|---|---|
| +3 SD | 31.0 | | | | | | |
| | | | | | | X | |
| | | | | | | | |
| +2 SD | 28.00 | | | | | | |
| | | | X | | | | |
| | | | | | | | |
| +1 SD | 25.00 | | | | X | | |
| | | X | | | | | |
| | | | | | | | |
| Mean | 22.00 | | | | | | X |
| | | | | | | | |
| | | | | | | | |
| -1 SD | 19.00 | | | X | | | |
| | | | | | | | |
| | | | | | | | |
| -2 SD | 16.00 | | | | | | |
| | | | | | | | |
| | | | | | | | |
| -3 SD | 13.00 | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Date | | 01-02-08 | 02-02-08 | 03-02-08 | 04-02-08 | 05-02-08 | 06-02-08 |

**BLANK LEVEY-JENNINGS CONTROL CHART**

**Instrument Identification:** _____

**Control Material Name :** _____

**Control Material Batch Number:** _____

**Control Material Expiration Date:** _____

**Analyte:** _____

| | Value | | | | | | |
|---|---|---|---|---|---|---|---|
| **+3 SD** | | | | | | | |
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| **+2 SD** | | | | | | | |
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| **+1 SD** | | | | | | | |
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| **Mean** | | | | | | | |
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| **-3 SD** | | | | | | | |
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| **Date** | | | | | | | |

**EXAMPLE PERSONNEL TRAINING RECORD**

**Trainee Name:** Jane Elsberry

**Trainee Job Title:** Practice Nurse/ Laboratory Nurse I

**Trainer Name:** Jessica Ann Moore

**Trainer Job Title:** Laboratory Director

| Task or SOP | Stage of Training or Competency (Trainer Initials/Date/Trainee Initials) | | | | Comments | Miscellaneous |
|---|---|---|---|---|---|---|
| | Trainee Observed | Trainee performed tasks with supervision | Trainee competent without supervision | Trainee competent to train others | | |
| SOP Chem 1.1 (Animed Chemistry Analyzer Operation) | JAM/02-02-05/JE | JAM/04-02-05/JE<br>JAM/05-02-05/JE | JAM/06-02-05/JE | JAM/09-02-06/JE | Quick learner! | Participate in next scheduled competency assessment on 01-09-2006 |
| SOP Chem 1.2 (Animed chem. Analyzer QC) | | | | | | |
| SOP Chem 1.3 (Apple Electrolyte Analyzer Operation) | | | | | | |
| | | | | | | |

**BLANK PERSONNEL TRAINING RECORD**

**Trainee Name:** _____

**Trainee Job Title:** _____

**Trainer Name:** _____

**Trainer Job Title:** _____

| Task or SOP | Stage of Training or Competency (Trainer Initials/Date/Trainee Initials) | | | | Comments | Miscellaneous |
|---|---|---|---|---|---|---|
| | Trainee Observed | Trainee performed tasks with supervision | Trainee competent without supervision | Trainee competent to train others | | |
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