SPECIAL REPORT

ASVCP quality assurance guidelines: control of preanalytical and analytical factors for hematology for mammalian and nonmammalian species, hemostasis, and crossmatching in veterinary laboratories

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Key Words
Instrumentation, laboratory management, method validation, personnel, specimen

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DOI:10.1111/j.1939-165X.2012.00413.x

Abstract: In December 2009, the American Society for Veterinary Clinical Pathology (ASVCP) Quality Assurance and Laboratory Standards committee published the updated and peer-reviewed *ASVCP Quality Assurance Guidelines* on the Society's website. These guidelines are intended for use by veterinary diagnostic laboratories and veterinary research laboratories that are not covered by the US Food and Drug Administration Good Laboratory Practice standards (Code of Federal Regulations Title 21, Chapter 58). The guidelines have been divided into 3 reports: (1) general analytical factors for veterinary laboratory performance and comparisons; (2) hematology, hemostasis, and crossmatching; and (3) clinical chemistry, cytology, and urinalysis. This particular report is one of 3 reports and provides recommendations for control of preanalytical and analytical factors related to hematology for mammalian and nonmammalian species, hemostasis testing, and crossmatching and is adapted from sections 1.1 and 2.3 (mammalian hematology), 1.2 and 2.4 (nonmammalian hematology), 1.5 and 2.7 (hemostasis testing), and 1.6 and 2.8 (crossmatching) of the complete guidelines. These guidelines are not intended to be all-inclusive; rather, they provide minimal guidelines for quality assurance and quality control for veterinary laboratory testing and a basis for laboratories to assess their current practices, determine areas for improvement, and guide continuing professional development and education efforts.

Introduction

Providing quality care to animal patients is an important goal for clinicians and often involves the use of clinical laboratory instruments and manual methods to generate clinically relevant data. Owing to the lack of governmental regulation of veterinary laboratory performance, veterinarians ideally should demonstrate a commitment to self-monitoring and regulation of laboratory performance from within the profession. The phases of the testing process are commonly divided into preanalytical, analytical, and postanalytical factors. Quality assurance at each phase of the

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

laboratory testing process serves to ensure that results are reliable and contribute to high-quality care of animal patients.

Preananalytical factors to consider include appropriate specimen identification, collection, handling, transport to the laboratory, and accessioning. Analytical factors include ensuring the precision, accuracy, and reliability of the testing process. ASVCP quality assurance guidelines related to the control of general analytical factors in veterinary laboratories have been published.[1] Postanalytical factors include data review, reporting and documentation, attention to timeliness, specimen storage, and cleaning and restocking supplies. This report documents recommendations for control of the preanalytical and analytical factors specifically related to hematology for mammalian and nonmammalian species, hemostasis testing, and crossmatching.

General Preanalytical Factors Important in Veterinary Clinical Pathology

Specimen collection, handling, and transport to the laboratory

Information concerning specimen requirements, proper collection, handling, and delivery or shipping procedures for any assay performed in the laboratory should be available to clients electronically, in written materials, such as a laboratory services manual, special information sheets, journal, or newsletter articles, or by personal telephone communication. Instrument manufacturer package inserts provide detailed descriptions of appropriate specimens, including collection tubes and handling conditions. Specimens should be collected according to standard practices and transported to the laboratory in a timely manner under conditions appropriate for the type of specimen and its stability. Type of specimen, eg, whole blood, serum, or plasma, should be clearly stated on the specimen label. Deviation from recommended protocols can adversely affect test results. Manufacturers can be contacted for specific details regarding adverse test results.

Specimens should be identified with pertinent information as determined by the laboratory, such as owner, animal identification, species and signalment, date of collection, name of clinic or veterinarian, address, telephone and fax numbers, e-mail address, and location from which the specimen was collected. Unique and matching identifiers should be located on both the submission form and container. The requested test(s) should be clearly marked or stated on the submission form.

Specimen accessioning

Specimen information, identification, and requested tests should be correctly entered into the laboratory information system (LIS); once entered, this information may be used to track the location and appropriate storage of the specimen, eg, hematology section or refrigerator. Specimen aliquoting and delivery to the appropriate section within the laboratory or between several departments should be coordinated. Any problems with specimen quality, including, but not limited to, hemolysis, lipemia, gelling of the specimen, or other preanalytical problems, should be recorded and reported to clients and laboratory staff. If the degree of inaccuracy associated with specimen quality is likely to affect clinical decisions, testing should not be performed on the specimen in question or results should not be reported. Problems with the reported or unreported results should be communicated to the client and, if possible, a new specimen obtained.

Client communication and education

Communication between laboratory personnel and clients (internal and external) should be timely and courteous regarding preanalytical factors, eg, incomplete submission forms, inappropriate specimen or specimen handling, or poor specimen quality, that may affect test results. Clients should be informed of the expected time for receipt of preliminary and final reports. Similarly, feedback from clients to the laboratory should be encouraged. All verbal or written complaints, feedback, and suggestions should be documented and forwarded to the appropriate level of management. Management meetings must be documented and organizational reviews conducted to ensure timely and appropriate follow-up on corrective actions.

Personnel safety and requirements

Conditions should be comfortable and appropriate for computer entry, data transcription, handling of specimens, specimen disposal, and all other tasks. Special consideration should be given for repetitive work. Personal protective equipment (PPE) should be appropriate for handling specimens and operating equipment in all areas of the clinical laboratory. Safety procedures for disposal of all specimens, waste, and other supplies should be appropriate for the type of material. Personnel should receive safety and biohazard training regarding exposure to potentially hazardous chemicals or infectious pathogens present in biological materials. Documentation of environmental, health, and safety training for each staff member should be available and

readily accessible. Training should include basic prevention of bacterial contamination as well as information on zoonotic diseases.

Laboratory environment

The laboratory environment should meet standard requirements for safe, rapid, efficient, and effective performance. The workspace should be well-lit and organized to promote efficiency and safety. Equipment and instrumentation should be in working order. Up-to-date procedure protocols should be easily accessible for reference when needed. Laboratory facilities and operation should be in compliance with appropriate government agencies.

Personnel requirements

Personnel should meet training requirements as indicated for specific areas of the laboratory. Training, continuing education, and recertification for specialized tasks should be regularly scheduled and documented. The laboratory should be staffed appropriately to meet the workload.

Laboratory information systems

An LIS is intended to improve work-flow and efficiency of the laboratory. Prior to implementation, the LIS should be thoroughly evaluated and the ability to maintain accurate records verified. An inefficient and unwieldy LIS should be updated or enhanced based on the needs of the laboratory. The LIS should meet all governing legal regulations for medical record archives. Problems with specimen accessioning or archival storage should be corrected immediately.

Hematology

Preanalytical factors

Blood films made in the clinic should not be refrigerated and should be protected from condensation and freezing during transport to the laboratory. Anticoagulated specimens for hematology that are found to have macroclots upon visual inspection will produce variably erroneous results. Because the degree of inaccuracy cannot be predicted, clotted specimens are unsuitable for analysis, and it is recommended that these specimens should not be analyzed. The clinic should be contacted either in writing or by phone and informed that the specimen will produce erroneous results. If specimens of questionable or substandard quality are analyzed, the laboratory should document in writing the nature of the quality issue and the possible resulting inaccuracies. In addition, any reports containing possibly inaccurate results should include obvious comments to the clinician that clearly state those values may be inaccurate and misleading.

Analytical factors

Quality control

Hematologic automated assays, calculated indices, and microscopic findings can and should be included in the quality control (QC) process whenever possible. Erythrocyte variables may include RBC concentration, hemoglobin (Hgb) concentration, hematocrit (HCT), manual PCV, RBC distribution width (RDW), MCV, MCH, MCHC, and reticulocyte count. Additional assays available only by direct laser measurement provided by some analyzers may include cell hemoglobin content (CH), cell hemoglobin content of reticulocytes (CHr), corpuscular hemoglobin concentration mean (CHCM), hemoglobin concentration distribution width (HDW), and mean cell volume of reticulocytes (MCVr).

Leukocyte and platelet variables may include WBC concentration or nucleated cell concentration (NCC), WBC differential counts (microscopic or automated), platelet concentration (PLT), mean platelet volume (MPV), platelet volume distribution width (PDW), and plateletcrit (PCT). Microscopic findings may include RBC and WBC morphologic appearance, number of nRBCs/100 WBCs, and estimated reticulocyte and PLT concentrations. Blood smears should be prepared, stained, and retained for microscopic examination at the discretion of the clinical pathologist. Criteria should be established for situations that require microscopic examination of the blood smear. Suggestions include confirmation of automated cell and differential counts at predetermined concentrations, eg, total WBC concentration > 20,000/μL. Cell counts performed manually using a hemacytometer must be performed in duplicate. If the difference between cell counts is > 10%, the chambers should be reloaded and counted again in duplicate. If a quality control material (QCM) is used for manual WBC, RBC, or PLT counts, either one level of assayed material or procedural control should be analyzed each time this method is used or once each shift. A procedural control is defined as duplicate aliquots of either an assayed QCM or a previously assayed specimen from an animal patient. Results may be compared with previously defined, acceptable limits for differences between

duplicates. This is the only acceptable procedural control for manual RBC counts.

WBC and PLT concentrations may be compared with a value estimated from a peripheral blood smear. New methylene blue-stained smears may be evaluated for a microscopic reticulocyte count. If the counts are performed in duplicate using 2 blood smears, the results should not differ by > 10%. Automated reticulocyte concentrations should correlate with the proportion of polychromatophilic RBCs observed on a stained blood smear. The PCV should approximate the HCT calculated by an automated analyzer using values for MCV and RBC concentrations. The laboratory should set the maximal acceptable difference, which may vary among species. MCHC may exceed the upper reference interval if hemolysis, occurring either in vivo or in vitro, lipemia, or large numbers of Heinz bodies are present. In the absence of these conditions, a high MCHC may indicate instrument error. Additional recommendations for QC are addressed in the general ASVCP quality assurance guidelines.[1]

Monitoring, method validation, instrumentation, personnel knowledge, procedures manual, and comparison of tests and outsourced tests

Monitoring recommendations for hematology are addressed in the general ASVCP quality assurance guidelines[1] and should include internal monitoring of all equipment with regard to electronic safety, calibration, maintenance, and performance. As not all the method validation procedures listed in the general guidelines are applicable to evaluation of automated hematology analyzers, method validation procedures should be selected or modified as necessary to ensure that new methods and analyzers are functioning satisfactorily to meet the laboratory's requirements and the manufacturer's specifications. Information about personnel knowledge, procedures manual, comparison of tests, and outsourced tests are important analytical aspects and are likewise addressed in the general guidelines.

Postanalytical factors

Specimens should be stored under appropriate conditions for a pre-established time period, as determined by specimen stability, laboratory policy, and certification and accreditation requirements. Stained microscopic slides may be held indefinitely, whereas specimens like whole blood have a limited storage life.[2–5]

Manual Hematology of Nonmammalian Species

Preanalytical factors

Acceptable transport times for avian blood specimens are shorter than that for mammalian and reptilian blood. Controlled studies have shown that refrigerated avian blood deteriorates within 12 hours regardless of anticoagulant,[6] whereas reptilian blood specimens are stable for 24 hours. Acceptable transport time for avian blood smears on glass slides is similar to reptilian and mammalian blood smears. Hematology specimens for shark species are less stable and should be processed within 5 hours due to cellular deterioration.[7] EDTA (7.5% or 1–2 mg/mL of blood) is acceptable for most, but not all, animal species. Blood from stingrays, some bony fish, and some avian species reacts atypically in EDTA, so heparin is commonly used for these species (J.A., personal communication). Blood from elasmobranchs (sharks, skates, and rays) should be placed in a tube containing dry anticoagulant due to their high plasma osmolality values (~1000 mmol/kg); liquid anticoagulants may be used if adjusted for osmolality.

Laboratory personnel should have specific training in handling and preparation of specimens from exotic species. Training should be documented and include basic prevention of bacterial contamination as well as information on zoonotic pathogens, including *Chlamydophila*, *Salmonella* spp., West Nile virus, avian influenza, and *Giardia*. Methods used to document training, continuing education, and periodic proficiency assessment should be at the discretion of the laboratory director.

Analytical factors

Monitoring, instrumentation, and personnel knowledge

Internal laboratory monitoring should include reagent preparation for cell-counting diluent (reagent grade water, verification of quality of new lot compared with that of previous lot). Equipment, eg, hemacytometers, weighted hemacytometer cover slips, hand tallies, calibrated pipettes, and differential cell counters, used for hematology procedures should be in good working order. For each piece of equipment, routine monitoring and regular maintenance, eg, annual calibration of pipettes and balances, should be performed and documented. Records of maintenance, malfunction, and repairs should be kept. Laboratory analysts must be proficient in cell identification for the species tested. Comprehensive knowledge of species variation when

using flow cytometry is important when verification by manual methods is required.

Method validation

All of the standard method validation procedures may not be applicable to evaluation of exotic manual hematology, and validation procedures should be selected or modified as necessary to ensure that new methods are functioning satisfactorily to meet the laboratory's requirements and the manufacturer's specifications. Abbott Diagnostics, Inc. (Abbott Park, IL, USA) has validated and supports automated cell counts for some nonmammalian species using the automated Cell-Dyn 3500 hematology analyzer (or higher model). Validation has been done at the laboratories of SeaWorld (Orlando, FL, USA) (J. Vandenberg, personal communication).

Quality control

Manual WBC counts using a hemacytometer are imprecise and have coefficients of variation (CV) ranging from 20 to 40%[6,8]; therefore, QC implementation and statistical analysis may indicate significant variation or lack of significant variation that is not relevant to daily operation. In method validation studies for shark species, CV was comparable to manual hematology for human WBC counts as reported in the B-D product insert for Unopette 365855 (Becton Dickinson and Company, Franklin Lakes, NJ, USA) when the specimen was processed within 5 hours of collection.[7] Currently, commercially prepared control materials are not available for nonmammalian blood cell counts. Procedural controls include duplicate aliquots of a specimen from an animal patient performed within the acceptable time limits for specimen stability and a WBC estimate from the blood smear. Each institution should document a reliable protocol for evaluating the accuracy of hemacytometer counts.[9] Estimated total WBC counts may be difficult due to the similar morphologic appearance of lymphocytes and thrombocytes when viewed at the lower magnifications typically used for WBC estimates of mammalian cells. Evidence of leukocyte or thrombocyte aggregation in the hemacytometer should be reported to indicate erroneous total WBC concentration and differential cell counts.

Proficiency testing (external QC) for technical staff members should be documented annually or more frequently, as determined by the institution. Testing should include comparison counts from the same blood specimen for total cell concentrations and for leukocyte differential counts. Specimen selection should be representative of the animal patient population, eg, avian species, reptilian species, teleosts, and elasmobranchs. Between laboratorians, hemacytometer counts should agree to within 15% and differential percentage results for each cell type should agree to within the 95% confidence interval.[10]

Quality control for direct cell count method — thrombocyte/lymphocyte error

Differentiation of thrombocytes and lymphocytes in the hemacytometer may be difficult for newly trained technologists, or for experienced technologists when counting cells from certain animal species. A good QC procedure is to count all nonerythrocytes in the 9 large squares of the chamber and calculate the total number (this is not the actual value and must be corrected based on the differential count). Perform the differential count twice on stained smears free of thrombocyte clumps: include thrombocytes in the first differential count and exclude them in the second. The latter is reported as the actual differential count. Using the percent thrombocytes from the first differential count and the total hemacytometer count, calculate the absolute value for thrombocytes and subtract it from the total nucleated cell count to determine the total WBC concentration. Example: Tally from hemacytometer with a 1:100 dilution = 750 nonerythrocyte cells; $750 \times 1.1 \times 100 = 82{,}500/\mu L$ for the total nonerythrocyte count. The first differential = monocytes (1%), lymphocytes (9%), heterophils (8%), and thrombocytes (82%); thus, the absolute value for thrombocytes = $0.82 \times 82{,}500 = 67{,}650$. Therefore, the total WBC concentration = $82{,}500 - 67{,}650 = 14{,}850/\mu L$.

Reagents and materials

Documented protocols for cell counts include direct counts using methyl violet[11] to determine total RBC, WBC, and thrombocyte concentrations, direct counts with no dye,[12,13] or an indirect method using phloxine B dye[14] for total WBC only. In the latter method, only the cells containing eosinophilic granules are stained and the total WBC concentration is calculated based on the percent heterophils and eosinophils from the differential. Reported method validation studies between the direct and indirect techniques are conflicting[9,15] and require further investigation, preferably following Westgard guidelines for method validation,[16] with a more representative number of animal species. The disparity in results may be due to the imprecision of each method. The diluent described by Natt and Herrick can be prepared in the laboratory and is suitable for all nonmammalian vertebrate species; however, when this diluent is used for some chelonian (turtle) and elasmobranch (sharks, skates, and

rays) species, additional salts are required to adjust the osmolality of the stock solution.[17] Hawkey's technique utilized the WBC Unopette (Becton, Dickinson and Company), which is no longer available. The Eosinophil Unopette 5877 (Becton, Dickinson and Company) with phloxine B diluent (Campbell's method) also is no longer available. Comparable diluents can be prepared by the user.

Primary and Secondary (Coagulation) Hemostasis Testing

Preanalytical factors

Most of the error (> 90%) associated with hemostasis assays may be attributed to preanalytical factors of transport and handling.[18–21] For this reason, a user-friendly flow chart was created that details important sampling techniques and that can be distributed to clients, veterinarians, and veterinary technicians (Appendix 1).

Compliance with specimen collection, storage, and transport requirements is mandatory for obtaining accurate test results. Whole blood should be collected in trisodium citrate anticoagulant in a 9:1 ratio and immediately mixed by repeated inversion. An accurate ratio typically is accomplished by filling to the indicated mark on the appropriate blood tube. Specimens that do not conform to this dilution should be rejected.[22] Citrate volume may need to be adjusted for specimens from very anemic and hemoconcentrated animals.[23] For tests requiring plasma, the plasma is separated from blood cells after centrifugation and transferred to a plastic, not glass, tube.[24,25] Whole blood and plasma specimen stability at ambient (15–22°C) or refrigerated (2–8°C) temperatures is 4 hours for activated partial thromboplastin time (APTT) and 24 hours for prothrombin time(PT). If testing is not performed within these time frames, plasma specimens should be stored at −20°C.[26] If specimens are mailed to a laboratory for testing, rather than directly transported, plasma should be placed in a plastic tube, frozen, packed on ice, and shipped to arrive within 24 hours. Fresh citrated whole blood, which is required for some coagulation instruments and for platelet function analyses, should be kept at room temperature (20–24°C) for < 1–2 hours.[27]

Analytical factors

Routine assays used for hemostasis testing may include platelet morphologic evaluation, concentration, and estimate; buccal mucosal bleeding time (BMBT); APTT; PT; fibrinogen concentration, and thrombin time (TT) or thrombin clotting time (TCT). More specialized tests may include platelet function tests that evaluate adhesion, aggregation, and secretion; platelet surface antibody tests; coagulation factor assays; and von Willebrand factor assays, including quantitative ELISA, collagen binding assay (CBA), and multimer analysis. Assays for DNA mutations, antithrombin activity, protein C and S concentrations, cell- and plasma-based coagulation (thromoboelastography), thrombin generation, and those evaluating fibrinolysis, such as fibrin(ogen) degradation products (FDPs) and d-dimer concentrations, are also available.

Quality control

The following QC recommendations are made with consideration of the labile time-sensitive nature of these assays. Individual laboratories must define hours of operation and shifts to accommodate coagulation assays. Thromboelastographic studies should be prearranged prior to the analysis as the instrument must be prewarmed and the assay run within a specific time interval after collection. Laboratory and point-of-care instruments should be calibrated regularly according to manufacturer recommendations. If electronic QC is available, it should be performed as recommended by the manufacturer. Statistical QC is recommended on a routine schedule and whenever there is a change in lot number of reagents or rotors, a change or repair of instrumentation, or any clinical concern. In general, at least one level of control material should be run for each shift in which a coagulation profile is requested. This may be performed prior to or concurrently with testing specimens from animal patients.

Results may be expressed as% of a pooled normal human or species-specific specimen (eg, antithrombin activity), and reference intervals are then provided as a % of pooled normal human or species-specific control, respectively.[28] Specimens from animal patients and species-specific control materials should be tested in duplicate.

Monitoring and method validation

Internal monitoring is more commonly performed because specimen instability may preclude external monitoring. Please see general recommendations for further description of internal monitoring. Not all the method validation procedures listed in the general ASVCP quality assurance guidelines[1] may be applicable to evaluation of coagulation analyzers. Method validation procedures should be selected or modified from the general recommendations as necessary to ensure that new methods and analyzers are functioning

satisfactorily to meet the laboratory's requirements and the manufacturer's specifications.

Crossmatching

Preanalytical factors

Serum or plasma may be used for crossmatching; however, the animal species and particular procedure may influence this selection. Fresh serum can serve as a source of complement in a procedure for detection of hemolysis in dogs and cats, but this is not typically performed. Specimens for major crossmatch include serum (nonadditive tube) or plasma (EDTA or citrated) from the recipient (animal patient) and anticoagulated (EDTA, ACD, or citrated) whole blood or packed RBCs from the donor(s). Specimens for minor crossmatch include anticoagulated whole blood from the recipient and serum or plasma from the donor(s). Recipient and donor specimens should be < 24-hours old when possible, although donor specimens from unit segments may be as old as the unit of blood to be crossmatched. If not used immediately, specimens should be stored at 4°C. For some procedures, whole blood is used, whereas others require a phosphate-buffered-saline washed RBC suspension.[29] General and specific recommendations for specimen collection, handling, and transportation of hematology specimens should be followed.

Specimens from the recipient and donor(s) should be clearly labeled as animal patient and donor(s) with date, time, and identification of the animal patient and each donor indicated on each specimen submitted. Specific forms for submission should be considered to ensure accurate assignment of specimens as recipient and donors.

Analytical factors

Quality control

Assays described in this section include major and minor crossmatching. A major crossmatch consists of testing patient serum or plasma with a saline suspension of donor RBCs. A minor crossmatch, available when a donor whole blood specimen has been submitted, consists of testing a saline suspension of RBCs form the recipient (animal patient) with donor serum or plasma.

If sera are used for crossmatching, sera should be separated from RBCs as soon as possible after the specimen has thoroughly clotted. Specimens should be examined for hemolysis and graded with 1+ being mild hemolysis and 4+ being severe hemolysis. Serum (or plasma) specimens with 3+ or 4+ hemolysis should be rejected, although more stringent rejection criteria of specimens with 1+ or 2+ hemolysis may be used. Hemolyzed serum or plasma may mask an incompatible hemolytic reaction when hemolysis is an indicator of incompatibility in the method being used.[30]

Autocontrols for the animal patient and donor(s) should be performed to ensure that reagents, such as the diluent, and equipment are functioning properly. Autocontrols should be handled in parallel with and identical to the major and minor crossmatch specimens. For the animal patient (recipient), the autocontrol consists of separated serum or plasma and a saline suspension of RBCs from the recipient. When donor whole blood is submitted, the donor autocontrol consists of donor separated serum or plasma and a saline suspension of donor washed RBCs.[31]

False positive results secondary to the following may occur:

- Strong rouleaux mimicking true agglutination; saline replacement can be performed to disperse rouleaux.[31]
- Inadequate washing.

False negative results secondary to the following may occur:

- Excessively dilute or concentrated RBC suspensions.
- Excessive shaking and tapping, which may disrupt fragile agglutinates.

Monitoring and method validation

Internal monitoring is more commonly performed as specimen instability may preclude external monitoring. Not all the method validation procedures listed in the general ASVCP quality assurance guidelines[1] may be applicable to evaluation of crossmatch methods. Method validation procedures should be selected or modified as necessary to ensure that new methods and analyzers are functioning satisfactorily to meet the laboratory's requirements and the manufacturer's specifications.

A procedures manual should be maintained and accessible to all laboratory personnel performing crossmatching. Procedures for crossmatch vary with the laboratory and species. Specific protocol recommendations are beyond the scope of these guidelines. Establishment of crossmatch procedures or adoption of procedures from another trusted laboratory is recommended.

Conclusions

Quality assurance and QC throughout all testing phases are essential in providing reliable and accurate results. Preanalytical and analytical recommendations specifically related to hematology of mammalian and nonmammalian species, hemostasis testing, and crossmatching are presented here. These and additional guidelines from the QAS Committee are available at www.asvcp.org under Publications and are revised and updated at regular intervals.[32] It is hoped that these guidelines and related publications will provide a basis for laboratories to assess their current practices, determine areas for improvement, and guide continuing professional development and education efforts.

Acknowledgments

This work is dedicated to the memory of Dr. Bernie Feldman, a former President of the ASVCP and initiator of the Quality Assurance and Standards (QAS) Committee formed in 1996, and Pat Benson, an early contributor, formerly at Texas A&M University. This work would not be possible without the hard work and persistent efforts of prior guideline contributors and reviewers. We gratefully acknowledge all individuals contributing to and reviewing these guidelines. Specifically, we thank the following (listed alphabetically): Kristin Barnhart (M.D. Anderson Cancer Center), Jim Bellamy (University of Prince Edward Island), Julia Blanco-Chavez (University of Guelph), Karen Curd (previously at Schering-Plough), Deborah Davis (IDEXX Laboratories), Rebecca Davies (University of Minnesota), Jean Dodds (Hemopet), Ellen Evans (Schering-Plough), Bente Flatland (University of Tennessee), Susan Friend (previously at Central Veterinary Diagnostic Laboratory, Australia), Ronna Fulton (FVClinPath Consulting), Susan Gallagher (University of Wisconsin-Madison), Urs Giger (University of Pennsylvania), Julie Gruenwaldt (IDEXX Laboratories), Rebekah Gunn-Christie (Antech Diagnostics), James Klaassen (Phoenix Central Laboratory for Veterinarians), Joyce S. Knoll (Tufts University), Helen Kocmarek (University of Guelph), David Korcal (Michigan State University), Peter Lording (University of Melborne), John Lumsden (University of Guelph), Mike Mahoney (previously at Oklahoma State University), Jim Matthews (IDEXX Laboratories), Larry McGill (Animal Reference Pathology), Joanne Messick (Purdue University), Pam Miller (previously at Antech Diagnostics), Fran Moore (Marshfield Laboratories), Loretta Moore (previously at Phoenix Central Laboratory for Veterinarians), Scott Moroff (Antech Diagnostics), Renee Pearson (Pearson Consulting), Shannon Pederson (Phoenix Central Laboratory for Veterinarians), Connie Peterson (Colorado State University), Kristiina Ruotsalo (University of Guelph), Karen Russell (Texas A&M University), Lynne Shanahan (Colorado State University), Carolyn Sink (Virginia/Maryland Regional College of Veterinary Medicine), Balazs Szladovits (The Royal Veterinary College), Gail Walter (Veterinary Clinical Pathology Consultant), Heather L. Wamsley (University of Florida), and Ellen Ziemer (Rougemont Preclinical Pathology).

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Appendix

COAGULATION SAMPLING GUIDELINES

SAMPLE QUALITY IS CRITICAL FOR VALID RESULTS.

- Blood must be drawn directly into sodium citrate anticoagulant and immediately inverted 8-10 times to mix (EDTA, heparin, serum separator, and clot activator tube samples are INVALID for coagulation assays).
- Avoid traumatic venipuncture, prolonged vessel occlusion, drawing blood into a dry syringe, incomplete blood draw, or air in collection tubing because these conditions may activate, deplete, or dilute coagulation factors.

VACUUM TUBE METHOD

1. Use vacuum collection needle or butterfly catheter to draw blood directly into a 3.2% or 3.8% citrate vacuum tube (blue top).
2. Make sure tube is in-date and filled to capacity by vacuum draw.
3. Blue-top tube should be filled after another tube or follow a discarded volume of blood to prevent tissue coagulation factor contamination from endothelium and air contamination from dry tubing.
4. Immediately invert tube 8-10 times to thoroughly mix anticoagulant and blood.

SYRINGE METHOD

1. Draw an exact volume of citrate into a syringe using 1 of the following examples:
   a. 0.2 mL citrate + 1.8 mL blood = 2.0 mL total sample
   b. 0.3 mL citrate + 2.7 mL blood = 3.0 mL total sample
   c. 0.4 mL citrate + 3.6 mL blood = 4.0 mL total sample
2. Perform venipuncture to collect total sample volume.
3. Remove needle and transfer blood sample to a plastic tube (not glass).
4. Immediately invert tube 8-10 times to thoroughly mix anticoagulant and blood.

PLASMA SEPARATION AND PROCESSING

1. Centrifuge clot-free blood specimen for 10 to 15 minutes at 1500*g*.
2. Harvest plasma promptly using plastic pipette or syringe and transfer to a clean plastic tube.
3. Store at room temperature and test within 4 hours or freeze for shipment.
4. If shipping, ship overnight on cold packs (or dry ice for special studies).

DO NOT FREEZE WHOLE BLOOD.

Modified from a handout by Dr. Marjory Brooks, Cornell University, 2009