

SPECIAL REPORT

Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries

Lindsay Tomlinson¹, Laura I. Boone², Lila Ramaiah³, Kelley A. Penraat⁴, Barbara R. von Beust⁵, Mehrdad Ameri⁶, Florence M. Poitout-Belissent⁷, Kurt Weingand⁸, Heather C. Workman², Adam D. Aulbach⁹, Dennis J. Meyer⁷, Diane E. Brown¹⁰, Amy L. MacNeill¹¹, Anne Provencher Bolliger⁷, Denise I. Bounous¹²

¹Drug Safety and Research Development, Pfizer, Inc., Cambridge, MA, USA; ²Covance Laboratories, Inc., Greenfield, IN, USA; ³Huntingdon Life Sciences, East Millstone, NJ, USA; ⁴Alpha-Omega Veterinary Consultant Services, Lafayette, NJ, USA; ⁵Labor am Zugersee, Hünenberg, Switzerland; ⁶Department of Pathology, Amgen, Inc., Thousand Oaks, CA, USA; ⁷Clinical Pathology, Charles River Laboratories, Reno, NV, USA; Montreal, QC, Canada; Sherbrooke, QC, Canada; ⁸SciComPro-LLC, Phoenix, AZ, USA; ⁹MPI Research, Mattawan, MI, USA; ¹⁰Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; ¹¹Department of Pathobiology, University of Illinois, Urbana, IL, USA; and ¹²Discovery Toxicology, Bristol-Myers Squibb Company, Lawrenceville, NJ, USA

Key Words

Biomarkers, laboratory standards, preclinical development, preclinical safety, translational medicine

Correspondence

Lindsay Tomlinson, Drug Safety and Research Development, Pfizer, Inc., Cambridge, MA, USA
E-mail: lindsay.tomlinson@pfizer.com

DOI:10.1111/vcp.12059

Abstract: The purpose of this paper by the Regulatory Affairs Committee (RAC) of the American Society for Veterinary Clinical Pathology (ASVCP) is to review the current regulatory guidances (eg, guidelines) and published recommendations for best practices in veterinary toxicologic clinical pathology, particularly in the pharmaceutical and biotechnology industries, and to utilize the combined experience of ASVCP RAC to provide updated recommendations. Discussion points include (1) instrumentation, validation, and sample collection, (2) routine laboratory variables, (3) cytologic laboratory variables, (4) data interpretation and reporting (including peer review, reference intervals and statistics), and (5) roles and responsibilities of clinical pathologists and laboratory personnel. Revision and improvement of current practices should be in alignment with evolving regulatory guidance documents, new technology, and expanding understanding and utility of clinical pathology. These recommendations provide a contemporary guide for the refinement of veterinary toxicologic clinical pathology best practices.

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. The recommendations contained in this manuscript have also been endorsed by the Society of Toxicologic Pathologists (STP).

Introduction

Summary of Best Practice Recommendations

Instrumentation, Validation, and Sample Collection

Good Laboratory Practices (GLP)

Laboratory Informatics and Management Systems (LIMS), Quality Control (QC), and Quality Assurance (QA) programs

Instrument and Method Validation

Blood Collection, Sample Collection and Handling – Pre-analytical Factors

Routine Laboratory Variables and Analytes

Hematology

(continued)

Coagulation
Clinical Chemistry
Urinalysis
Non-routine Variables and Analytes
Cytologic Specimens
Broncho-Alveolar Lavage
Other Body Fluids (cerebrospinal fluid, synovial, pleural, and abdominal fluids)
Bone Marrow Cytology
Data Interpretation and Reporting
Data Interpretation
Data Reporting
Peer Review
Reference Intervals
Veterinary Clinical Pathologist and Laboratory Personnel
Veterinary Clinical Pathologist
Expanding Roles for Veterinary Clinical Pathologists
Translational Research and Medicine
Clinical Pathology in Clinical Development
Laboratory Professional Staff
Conclusions
Acknowledgments
References

Introduction

Veterinary clinical pathology is a specialty of growing recognition in product development for animal and human health industries. The use of appropriate and validated instruments and assays operated and performed according to standard operating procedures (SOPs) by adequately trained personnel is crucial to the production of high-quality clinical pathology data. Inclusion of guidance-driven and specific pertinent routine and nonroutine clinical pathology variables and analytes ensures that relevant project data are available for evaluation. An integrative interpretation of high-quality relevant data is required to produce a high-caliber product.

Veterinary clinical pathologists' contributions to professional societies and the literature involving the pharmaceutical and biotechnology industries demonstrate that clinical pathology expertise assists with the refinement, standardization, and optimization of practices within industrial clinical pathology laboratories. Ultimately, these contributions lead to rigorous data generation and interpretation.

Guidances provided by regulatory agencies, such as the Organisation for Economic Co-operation and Development (OECD), United States Food and Drug Administration (FDA), European Medicines Agency (EMA), Ministry of Health, Labour and Welfare (MHLW), and Environmental Protection Agency (EPA), provide the framework for inclusion and evalu-

ation of clinical pathology in routine toxicology studies performed for pharmaceutical and biotechnology drug development and for the agrochemical industry. Publications in peer-reviewed journals, including position papers of associations such as the American Society for Veterinary Clinical Pathology (ASVCP), Society of Toxicologic Pathologists (STP), and American Association for Clinical Chemistry-Division of Animal Clinical Chemistry (AACC-DACC), have influenced the regulatory environment for clinical pathology testing and have been used in specific cases to address modified testing approaches. Some flexibility or a "fit-for-purpose" approach based on the class and type of test article and clinical regimen being evaluated is considered appropriate, and is built into both the regulatory guidances and the positions of societies such as ASVCP, STP, and AACC-DACC. The Regulatory Affairs Committee (RAC) of the ASVCP reviewed the guidances and the literature regarding veterinary clinical pathology in the pharmaceutical and biotechnical industries. ASVCP RAC's objective was to determine which practices remain contemporary and which areas require clarification or updating to provide current best practice recommendations and references for the industries involved.

With the regulatory framework in mind, this review discusses recommendations for (1) instrumentation, validation, and sample collection, (2) routine, and (3) cytologic laboratory variables, (4) data interpretation and reporting, peer review, reference intervals and statistics, and finally, (5) roles and responsibilities of veterinary clinical pathologists and laboratory personnel, as they pertain to industry, with emphasis on the pharmaceutical and biotechnology industries.

Summary of Best Practice Recommendations

Instrumentation, validation, and sample collection

Good laboratory practices (GLP)

While laboratory testing of clinical (human) specimens must comply with the Clinical Improvement Act of 1988 (CLIA), laboratories testing nonclinical or pre-clinical (ie, nonhuman studies supporting human or veterinary clinical trials) samples in support of FDA/EPA/OECD applications are required to comply with Good Laboratory Practices (GLP). The GLP standards, including the FDA Code of Federal Regulation 21 Part 58, EMA Note for Guidance on Good Clinical Practice, and OECD Principles of Good Laboratory Practice,¹⁻³ describe required standard practices concerning valida-

tion, certification, and/or qualification of all operations including personnel, facilities, systems, equipment, methods, reagents, sampling, sample handling, sample testing, stability testing, and raw data management. In addition, documentation, review and approval of test results, data recording and reporting, as well as plans for corrective action, risk management, and disqualification of testing facilities, are addressed.

In general, all laboratory activities should be traceable and accountable ie, who did what and when, and documented in either hard copy or electronic form. Compliance with GLP requirements is directed by the use of SOPs, which are maintained by the laboratory. Standard operating procedures should be written in a self-explanatory manner outlining procedures that are easy to follow for adequately trained technical personnel. All technical personnel in the laboratory are required to obtain regular documented training in SOPs relevant for their assigned activities.

Clinical pathology laboratories not evaluating samples from GLP studies, or non-GLP certified laboratories, should maintain practices that are as close to GLP standards as appropriate. Best practices in the clinical pathology laboratory described in this manuscript are acceptable under GLP standards and are recommended for all laboratories, regardless of GLP status.

Laboratory informatics and management systems (LIMS), quality control (QC), and quality assurance (QA) programs

Laboratory informatics and management systems (LIMS) for the identification, assignment, collection, management, transfer, analysis and storage of electronic data (results and QC) and records should be validated according to the respective guidances.^{4,5} The authenticity of data collection by interface with an analytical instrument and the data transfer should be tested and documented. This includes the verification of the assignment of a particular sample to unique bar code identification. In addition, computer access safety (unique and personal User ID and password) needs to be tested and documented. Finally, the safe archiving of study and instrument data needs to be validated. Archived data must be retrievable at any time, but must be protected from editing (ie, read only).

Whether data and records are stored on paper or electronically, the audit trail should disclose all instrument-related activities, interactions, and events: date and time when sample measurement was performed, identity of the instrument, and personnel who ran the samples and signed as responsible for the raw data. Any modification or editing of data must be documented with day and time of change, identity of the person performing the change, and

reason for the change, if applicable (eg, outliers due to preanalytical or analytical parameters). Likewise, all changes to an instrument's original set up must be explained and documented with the date and the person responsible for the change.

We refer the reader to ASVCP Quality Control Guidelines documents,⁶⁻⁸ also available at www.asvcp.org, for a thorough review of QC requirements and recommendations in the nonclinical laboratory setting. Briefly, QC generally ensures consistent practices in the laboratory associated with generating and reporting quality data, whereas QA refers to audit or verification processes performed by an independent QA group that ensures compliance with GLP regulations.

Internal QC includes specific acceptance and rejection criteria for each assay. This is performed on instruments either at the beginning of a working shift or before and after studies, depending on the laboratory workflow and volume. In addition, QC is performed any time reagents are changed and following any instrument maintenance (calibration, cleaning, trouble-shooting) during a working shift. Internal QA consists of planned and systematic monitoring to ensure that facilities, equipment, personnel, methods, practices, records, and controls conform to applicable regulations and to verify that data generated meet specific set expectations. The use of computer software to harness and report QC results is not specifically regulated by GLPs. However, available instrument QC software for general LIMS is available from companies such as Xybion (Morris Plains, NJ, USA), Rhoads EP Evaluator (Kennett Square, PA, USA), and BioRad (Hercules, CA, USA). The use of LIMS instrument QC software is recommended by the ASVCP RAC as it increases laboratory efficiency and reduces the risk for manual error.

External QC includes participation in external proficiency programs (human or veterinary) that may be specific to veterinary diagnostic laboratories. This usually entails analysis of a common material among all participating laboratories followed by comparison of closeness of individual laboratory results to the group mean. Although participation in external QC is generally voluntary, when available, ASVCP RAC recommends external QC measures appropriate for the species being evaluated to provide added assurance that internal processes are consistent with those of the industry at large.

External QC measures commonly employed in the USA include College of American Pathologists (CAP) and BioRad surveys as well as the veterinary specific, Veterinary Laboratory Associations (VLA) quarterly proficiency surveys. Other external QC options, which

are not veterinary specific, are available, but may be country or region specific and include R&D Systems, National External Quality Assessment Scheme (NEQAS), Randox International Quality Assessment Scheme (RIQAS), Roche QC, Institute for Hygiene and Epidemiology (IHE), and Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek (SKML).

Instrument and method validation

Validation of new instruments must be performed prior to reporting results to establish the suitability of an instrument and associated reagent systems. This validation must be performed at the facility where the instrument is to be used. Instruments with adjustable settings for different substances and/or species should be carefully checked for compliance, and accommodation of species differences must be ensured. Laboratory- and manufacturer-defined performance characteristics should be compared and adjustments made as needed.⁶⁻⁹

To ensure satisfactory function, critical operating characteristics of all instruments such as stray light, zeroing, electrical levels, optical alignment, and background checks should be assessed. Instruments should be calibrated according to the manufacturer's instructions and at a minimum, every 6 months. More frequent calibrations may take place, such as following a major service, when quality control values are outside limits, or when workload, equipment performance, or reagent stability indicate the need for more frequent calibration. After calibration, controls should be run according to SOPs.⁹ Because instrument validation is closely tied to method validation, the remainder of the section will address method validation.

For the purpose of this paper, method validation refers to "assessing the performance characteristics of a given assay,"¹⁰ rather than validation that specifically complies with Good Laboratory Practice regulations.¹ Method validation is recommended for any assay performed in the clinical pathology laboratory and should include an appropriate level of validation for the particular analytical method.

The number and variable types of assays (including immunologic methods to evaluate potential biomarkers) and applications in nonclinical studies preclude a standardized process for validation. While the American Association of Pharmaceutical Scientists (AAPS),¹¹⁻¹⁶ the FDA,¹⁶ and the EMA¹⁷ formulated approaches to validate assays for nonclinical studies, the guidances and recommendations are for bioanalytical method development and are not specific for clinical pathology assay validation. This discussion provides a brief overview of the basics of assay validation and is

specific for clinical pathology in nonclinical studies, but will not specifically cover method comparison.¹⁸ The proposed approach is termed "fit-for-purpose" as the intended use of the assay (eg, research, drug discovery, animal model development, efficacy, nonclinical, or clinical studies) will determine the rigor of assay validation.^{10,19} Assays used in early drug discovery often support a go/no-go decision for further drug development rather than for human risk assessment or regulatory submissions. Therefore, assay validation for early drug development/discovery can be limited to preliminary validation that demonstrates the performance of the assay, yet minimally impacts resources and the drug development timeline.

Factors to consider in assay validation under this paradigm consist of (1) supported studies (nonclinical or clinical), (2) sample matrix, (3) anticipated range of the variable or analyte in the assay, (4) potential interfering substances in the matrix, (5) acceptable level of variability in the results, and (6) availability of reagents and technology. The method should be validated to ensure that its performance conforms to the standards of the laboratory and claims of the manufacturer.

Assay validation requires the selection of the intended biological matrix, including the potential effects of interfering substances and dilution. Evaluations for assay validation include (1) accuracy, (2) calibration curve, (3) precision, (4) total error, (5) lower and upper level of quantification (LLOQ and ULOQ, respectively) to determine assay range (LLOQ - ULOQ), (6) sensitivity, (7) limit of detection (LOD), (8) dynamic range (range of linearity), (9) specificity, and (10) analyte stability.^{11,13,14,16,19-23}

Accuracy refers to the closeness of agreement between the mean value, from repeated determinations at an expected analyte concentration, and its true/known (nominal) concentration. It is expressed as percent recovery or relative error (RE). Published criteria for accuracy are: (1) the observed concentration should have an RE of $\leq 20\%$ of the nominal concentration, with the exception of the LLOQ and ULOQ where the RE should generally be $\leq 25\%$ or (2) recovery between 80% and 120% of the nominal concentration.^{13,22}

Calibration (standard) curve represents the relationship between the instrument response and the nominal concentration of the analyte. In general, a calibration curve should (1) be generated for each analyte of interest, (2) utilize a sufficient number of calibrators (often provided by the manufacturer) to define the relationship between the signal and the nominal concentration, and (3) be generated from spiking known concentrations of the standard/

calibrator into the intended biological matrix. The ideal matrix should be the same as that intended for the test samples and characterized by (1) a low background signal, (2) negligible analyte activity, (3) the absence of interfering substances, and (4) proportionality of the concentration of the spiked calibrator to the low background or nominal concentration of the analyte.

The calibration curve should consist of a blank matrix sample, a zero sample (matrix containing a negligible/background analyte concentration), and a suitable number of nonzero samples to cover the anticipated analytical range, analyzed in duplicate or triplicate. Published recommendations for the appropriate number of nonzero samples for immunoassay validation vary, but generally range between 5 and 9 samples.^{11,13,14,16,19–23} At least 75% of the calibrator standards of the calibration curve should be within the predetermined performance criteria. The calibration curve is then fitted to the mean of the replicates at each calibrator concentration using the most appropriate model that describes the concentration–response relationship.²⁴

Precision is the closeness of agreement of the results from multiple sampling of one specified sample. Precision is expressed as the standard deviation (SD) or coefficient of variation (CV) and quantifies the random error of the assay (intra-assay [“within-run”], interassay [“between runs”], and repeatability [between laboratories]). Precision should be determined using a minimum of 3 analyte concentrations within the range of expected concentrations, as predicted from the calibration curve, with a minimum of 5 determinations per concentration. Published guidances for precision generally state that the precision at each concentration should be 15–20% of the CV except at the LLOQ where it should be 20–25% of the CV.^{11,12,16,21,23}

Total error is the sum of accuracy/recovery and precision associated with the obtained result and should not exceed 30% of the CV. The LLOQ and ULOQ will be the lowest and highest acceptable standard concentrations on the calibration curve, respectively, that meet the predetermined criteria for accuracy and precision. The selection of the LLOQ is important as a high LLOQ results in decreased sensitivity (see below), but a low LLOQ may result in overlap with the blank matrix (background) response.

Sensitivity refers to the lowest concentration of an analyte that can be reliably differentiated from background by the analytical method. The limit of detection (LOD) concentration is one that can be reliably detected, but does not generally meet the criteria for accuracy, and can be differentiated from back-

ground with a certain degree of statistical certainty, as defined by a probability of differentiation of 95%. Data collected between the LOD and LLOQ should be interpreted with caution due to the higher variability within this range. Therefore, it is recommended that concentrations below the LLOQ are not reported.

The dynamic range (range of linearity) of the assay is the concentration span between the LLOQ and ULOQ with acceptable levels of accuracy, precision, and total error.²⁵ Concentrations that exceed the ULOQ require dilution to obtain results within the linear range of the assay.

Specificity refers to the ability of an analytical method to quantify and differentiate the analyte of interest from other analytes or variables. Nonspecific assays can generate false positives and/or overestimation of the variable of interest.

Evaluation of stability determines the integrity of the analytes(s) in matrix under all potential sources of preanalytical variability including sample collection and handling, storage at the intended temperatures and conditions, following freeze and thaw cycles, and during analysis. If proteolytic degradation is likely to impact stability of the analyte, then samples can be mixed with a protease inhibitor and kept on ice if analysis will occur within a few hours, or frozen.

ASVCP RAC recommends that best practices for method validation utilizing large automated instruments should at minimum include the following evaluations: accuracy, precision, sensitivity, and dynamic range. When more manual methods (eg, immunological methods) are being validated, total error, LLOQ, ULOQ, LOD, and specificity should also be evaluated. Further validation procedures may be implemented, based on the intended purpose of the assay, quality standards of the laboratory, and the stage of drug discovery that will be supported by the data generated. ASVCP RAC also recommends the best practice of establishing the stability of each analyte for each species tested in the laboratory and under the conditions that it will be evaluated, when possible.

Blood collection, sample collection, and handling – preanalytical factors

Specimens should be collected from animals using consistent and reproducible methods, and with consideration for fasting status. Specimen tubes must be labeled appropriately with specimen type (eg, whole blood, serum, plasma, or urine), animal identification, and date/time of collection. Blood collection site and method, means of restraint, and experience of handler and/or collector may impact the results. For example, anesthetic agents used to immobilize or minimize

an animal's stress during blood collection must be carefully considered as these can effect physiologic changes²⁶ that can impact test results.^{27–29}

Blood collection sites vary among laboratory animal species. In dogs, a common collection site is the jugular vein; however, the cephalic, saphenous, or other peripheral vein may also be used. Common collection sites for nonhuman primates (NHP) are the femoral vein and artery; however, the saphenous, cephalic, and cubital veins can also be used. A common site of blood collection in the rabbit is the ear vein or artery; other possible sites include the saphenous vein, jugular vein, or heart (terminal collection). The ante-mortem sites for blood collection in rats include the jugular vein, tail vein, retro-orbital sinus (with anesthesia), and sublingual vessel. In mice, because of potentially severe adverse effects, the retro-orbital site for ante-mortem blood collection has been largely replaced by other sites such as the submandibular vein.³⁰ Peri-mortem collection sites for rodents include the vena cava, heart, or aorta.²⁸ The acceptable blood withdrawal volume and frequency for nonterminal collections is dependent on the animal's circulating blood volume, which is based on a number of factors, including species, age, health condition, body weight, frequency, and method of blood collection.²⁸

EDTA (forms include Na₂EDTA, K₂EDTA, and K₃EDTA) is the preferred anticoagulant for hematology samples. K₂EDTA, which is spray-dried on the sides of blood collection tubes, will not dilute the sample, and is recommended by the International Council for Standardization in Haematology (ICSH) and the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Laboratory Standards) for hematology testing.^{31,32} To ensure thorough mixing of blood with anticoagulant, and to minimize clotting, the tubes should be gently inverted. Use of a tube rocker will ensure that samples remain well mixed following collection and prior to analysis. Most hematology variables are stable at 4°C for up to 24 hours.^{33–35} Fresh blood smears should be prepared immediately, but at a maximum, within 6 hours of phlebotomy.^{35,36}

For coagulation specimens, whole blood should be collected in trisodium citrate anticoagulant at a 9:1 ratio (blood:anticoagulant). Plasma should be separated from blood cells as soon as possible by refrigerated centrifugation (a minimum of 10 minutes at approximately 2000*g*).³⁷ If testing is not performed immediately, plasma samples can be frozen at –60 to –80°C.³⁸ However, depending on species and laboratory, long-term storage of citrated plasma samples may affect prothrombin time (PT) and activated partial thromboplastin time (APTT).³⁷

For serum chemistry analyses, blood should be collected into tubes without anticoagulant. Blood samples should be allowed to clot at room temperature for at least 30 minutes.³⁹ Samples should be centrifuged for about 10 minutes at 1000–2000*g*, serum separated and, if not analyzed immediately, stored at –60 to –80°C. If serum is not available, lithium heparinized plasma may be used for clinical chemistry analyses, but only if validation for all analytes has been performed using lithium heparinized plasma. Lithium heparin is preferred to other salts of heparin, such as sodium, because it is least likely to interfere with testing of other ions. Use of anticoagulant should be generally consistent across studies.

Urine is most often collected overnight (12–16 hours) in collection pans or metabolism cages. Urine may also be collected by free flow/voiding (mid-stream, early, or late), catheterization, or cystocentesis. Because analyte stability in urine depends on the variables of interest, broad recommendations for urine storage cannot be made.⁷ For extended collection periods, however, urine samples may be kept chilled (ie, collected over wet ice) to avoid bacterial growth or analyte degradation, bearing in mind that cooling of urine may result in crystal precipitation. Urine preservatives may interfere with some tests and should be avoided for routine analyses.

Routine laboratory variables and analytes

The core clinical pathology panels recommended by AS-VCP RAC (Tables 1–4) for nonclinical toxicity studies consist of well-characterized hematology and coagulation, clinical chemistry and urine analyses that provide critical information about general body homeostasis and organ function and/or injury. Minimum testing recommendations for nonclinical repeat-dose toxicology or safety studies have been previously published.⁴⁰ The previously published recommendations from 1996 represent the consensus of a joint international scientific committee, and include a list of variables and analytes that may be modified based on study objectives, design and duration, test article, species, biological activity of the test article, and regulatory guidances. Platform and reagent accessibility may also influence test selection. In addition, tests included on a minimum or core panel should be pathophysiologically relevant, validated, and have available historical reference data in common laboratory animal species.³⁹

While regulatory agencies and international organizations provide guidances and recommendations for testing, these vary among agencies, are updated infrequently, and may be vague. Regulatory agencies gen-

Table 1. ASVCP RAC-recommended hematology variables for routine toxicology studies* in the dog, cat, nonhuman primate, minipig, rat, mouse, rabbit, guinea pig, and hamster.

Hematology Variables	
HCT	WBC, total and differential counts†
HGB	Blood Smear Collection
RBC	Bone Marrow Smear Collection‡
MCH	
MCHC	
MCV	
RDW	
Reticulocyte count†	

*Excluding standard 2-year carcinogenicity studies in rodents.
 †Absolute counts are recommended over percentages.
 ‡Bone Marrow Smear Collection recommended in regulated studies and carcinogenicity studies on a case-by-case basis.

Table 2. ASVCP RAC-recommended coagulation variables for routine toxicology studies* in the dog, cat, nonhuman primate, minipig, rat, mouse†, rabbit, guinea pig, and/or hamster.

Coagulation Variables	
PT	Platelet count†
APTT	Fibrinogen

*Excluding standard 2-year carcinogenicity studies in rodents.
 †The only variable recommended for mouse; routinely included in hematology.

erally recommend a flexible approach, depending on species and the observed and/or expected effect of a given substance, and explicitly state that additional testing may be necessary to extend the search for toxic effects or elucidate toxic effects attributable to a test article.^{41,42} A flexible approach also ensures that test usage remains relevant over time, particularly if guidances pre-date the currently available standard technology.

The EMA makes no stand-alone recommendations for clinical pathology, but rather refers readers to

Table 3. ASVCP RAC-recommended serum chemistry analytes for routine toxicology studies* in the dog, cat, nonhuman primate, minipig†, rat, mouse, rabbit, guinea pig, and/or hamster.

AST	Total cholesterol
ALT	Triglycerides
Sorbitol dehydrogenase† and/or glutamate dehydrogenase†	Glucose
ALP	Urea
GGT‡	Creatinine
Total bilirubin	Total calcium
Total protein	Phosphorus
Albumin	Sodium
Globulin	Potassium
Albumin:globulin ratio	Chloride

*Excluding standard 2-year carcinogenicity studies in rodents.
 †Recommended specifically for minipig.
 ‡Recommended specifically for nonrodent species.

Table 4. ASVCP RAC-recommended urinalysis variables and analytes for routine toxicology studies* in the dog, cat, nonhuman primate, minipig, rat, guinea pig, and hamster.†

Volume	Glucose
Color	Protein
Clarity	Blood
Specific Gravity	Ketones
pH	Bilirubin

*Excluding standard 2-year carcinogenicity studies in rodents.
 †Mouse excluded due to volume limitations and rabbit excluded due to high urine turbidity.

recommendations made by a Joint Scientific Committee for International Harmonization of Animal Clinical Pathology Testing.⁴⁰ Some FDA and EMA recommendations exceed those of Weingand et al.⁴⁰ For example, clinical pathology testing for carcinogenicity studies or rodent studies longer than 52 weeks is not recommended by Weingand et al., or by an STP/ASVCP working group.⁴³ However, the EMA note for guidance on carcinogenic potential⁴⁴ and FDA’s guidance on safety assessment of food ingredients specific to rodent carcinogenicity⁴⁵ indicate that clinical pathology testing should be performed to maximize the information obtained from these studies. The ASVCP RAC recommends the position of the Carcinogenicity Study Working group (STP/ASVCP)⁴³ because the clinical pathology toxicity profile is better determined in general toxicity studies where age-related changes are not confounding to interpretation.

Hematology

Standard hematology testing recommended by FDA⁴¹ and OECD⁴² includes HGB, Weingand et al⁴⁰ also recommends routinely evaluating absolute reticulocyte counts and blood smears.

Although the FDA and OECD suggest that reticulocyte counts need not be routinely reported except in studies with test articles known to have an effect on

the hematopoietic system,^{41,42} ASVCP RAC also recommends reporting them. Advances in automated reticulocyte counting and increases in test reliability and availability correlate with increased usage of this variable across industry since the time of publication of applicable regulatory guidances.

Historically, reticulocytes and WBC differentials were reported as percentages because they were derived from manual counts performed on blood smears. Because absolute numbers more accurately define clinically relevant changes, differential counts should be reported as absolute numbers. Percentages may be useful to classify leukemic cells (blast percentage) observed on manual smear examination, but otherwise, evaluation of percentages is not recommended.^{39,46} For both reticulocyte counts and WBC differential counts, the ASVCP RAC recommends reporting the results in absolute numbers rather than in percentages.

The ASVCP RAC recommends that blood smears be routinely prepared, but not evaluated unless considered necessary for evaluation of cell morphology, platelet clumping, left shifts to immature cells, and presence of nucleated RBCs, as well as confirmation of automated counts and/or other variables obtained from the automated analysis. Criteria for slide evaluation should be defined in SOPs based on species-specific triggers, critical limit values, and/or instrument limitations. Although not routinely evaluated, bone marrow smears are often prepared at necropsy so that they are available for evaluation if indicated (refer to Bone Marrow Cytology).

Newer tests available with updated automated hematology analyzers may be useful, particularly during drug discovery or as adjuncts to investigation of RBC and platelet abnormalities. However, these variables, which include CHCM (cell hemoglobin concentration mean), MPV (mean platelet volume), HDW (hemoglobin distribution width), PDW (platelet distribution width), CHr (reticulocyte hemoglobin content), and MCVr (reticulocyte mean cell volume) are not recommended for standard testing at this time.

The ASVCP RAC recommendation for core hematology testing in routine toxicology studies is RBC, HGB, HCT, MCV, MCH, MCHC, RDW, absolute reticulocyte count, WBC, and absolute WBC differential counts (Table 1). RDW has been added to provide information on the variability of RBC size, which is corroborative of changes in MCV, and is routinely generated by automated analyzers.

Coagulation (Hemostasis)

For the purposes of this discussion, coagulation is not limited to the evaluation of the coagulation cascade

and in vitro fibrin formation, but also includes the role of platelets in forming a blood clot. Hemostasis includes clot formation as well as fibrinolysis and anti-coagulant proteins. Weingand et al.⁴⁰ recommend the evaluation of PT, APTT, and platelets. The FDA⁴¹ recommends that clotting potential be measured using one of the following: PT, APTT, clotting time, and/or platelet count. The OECD recommends that PT, APTT, and/or clotting time be measured, depending on the length and type of study.⁴² In addition to PT, APTT, and platelet count, fibrinogen is often measured because it is an acute phase plasma protein critical to blood clotting. The ASVCP RAC recommends that PT, APTT, platelet count, and fibrinogen be measured routinely when blood volume is not limiting (Table 2). In mice, for example, the evaluation of coagulation may be limited to platelet count.

Additional tests of hemostasis, which include all aspects of blood clot formation and breakdown, such as thrombin time or thrombin clotting time, anti-thrombin III, fibrin degradation products, D-dimer, plasminogen activator inhibitor-1 (PAI-1), P-selectin, thrombin antithrombin, Factor VIIa, prothrombin fragment 1+2 (PF1+2), platelet function/aggregometry, thromboelastography, and flow cytometry for specific markers, can be considered on a case-by-case basis. The HESI Cardiac Biomarkers Working Group has an ongoing effort to provide more specific recommendations on how to utilize additional markers of hemostasis and to close a potential gap in the risk assessment for hypercoagulable states.⁴⁷

Clinical chemistry

Guidances and task force consensus^{40,41} recommend that the clinical chemistry panel includes assessments of glucose, urea, creatinine, total protein, albumin, calculated globulins, total calcium, sodium, potassium, total cholesterol, and appropriate hepatocellular and hepatobiliary evaluations. For the detection of hepatocellular injury, a minimum of 2^{40,42,48} or 3⁴¹ of the following tests have been recommended: ALT, AST, sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH, alternatively known as GLDH), and total bile acids. For the detection of hepatobiliary injury, at least 2^{40,42} or 3⁴¹ of the following should be included: ALP, total bilirubin, GGT, 5' nucleotidase and total bile acids. The reader is referred to a recent publication delineating recommendations for the use of clinical pathology end points in assessment of the potential for drug-induced hepatic injury in nonclinical studies that include measurement of ALT, AST, ALP, total bilirubin, direct bilirubin, total protein, and albumin.⁴⁹ ASVCP RAC recommends evaluation of direct bilirubin

only if relevant increases are seen in total bilirubin, as determined by each laboratory.

In addition, FDA stipulates that additional clinical chemistry evaluations may be recommended to ensure adequate toxicological evaluation of the test article. These include analyses of acid/base balance, hormones, lipids, methemoglobin, and proteins.⁴¹ OECD guidances indicate that in mice, satellite animals may be needed to conduct all required clinical chemistry evaluations.⁴²

Analytes that may be evaluated in all or limited species are GGT, SDH, GDH, CK, indirect bilirubin, bicarbonate or total CO₂ (HCO₃ or TCO₂, respectively), and magnesium. For example, because of the relatively low ALT activity and poor liver specificity in minipigs, SDH and/or GDH are recommended in addition to AST and ALT.^{50,51} CK may be of particular use in evaluating muscle injury in dogs.⁵² For a more thorough evaluation of electrolytes or acid/base status, inclusion of magnesium or bicarbonate, respectively, may be considered. In studies conducted for veterinary drug development, the clinical chemistry analytes may need to be adapted for the species of interest.

The ASVCP RAC recommendation for a core clinical chemistry panel in routine toxicology studies includes ALT, AST, ALP, and GGT (GGT is recommended specifically for nonrodent studies), total protein, albumin, globulins, albumin:globulin ratio (A/G), cholesterol, triglycerides, glucose, urea, creatinine, total bilirubin, total calcium, potassium, phosphorus, sodium, and chloride, with the addition of SDH and/or GDH in minipigs (Table 3).

Urinalysis

The FDA Redbook⁴¹ and Weingand et al.⁴⁰ recommend that urinalysis include measurements of urine volume, specific gravity, pH, glucose, and protein. Weingand et al. also specify the inclusion of appearance, the option to replace specific gravity with osmolality, and that analysis of total protein and glucose may be quantitative or semi-quantitative. Importantly, while the FDA Redbook recommends microscopic evaluation of urine sediment, Weingand et al. indicate that microscopic examination and electrolyte excretion are not recommended for routine studies. For urine sediment microscopy, the numerous sample quality challenges (ie, stability and contamination) impact the benefit of this analysis. However, ASVCP RAC proposes that properly handled sediment microscopy should be considered when toxicology and pharmacology are indicative or predictive of urinary tract changes.

Reagent test strips used as a semi-quantitative screen for pH, protein, glucose, ketones, bilirubin and

blood have the advantages of speed, low cost, low volume requirements and translatability to the clinic. However, the tests are designed for use with human urine and have a number of limitations in animal species. For example, urine specific gravity, urobilinogen, nitrites, and/or leukocytes are included on some reagent strips, but these are not useful or recommended for animal samples.^{53–58}

ASVCP RAC recommendations for routine urinalysis variables and analytes for dog, NHP, minipig, rat, guinea pig and hamster include volume, color, clarity, specific gravity or osmolality, pH, blood, ketones, bilirubin, protein, and glucose (Table 4). The panel is expanded to include semi-quantitative analysis of blood, ketones, and bilirubin, as these are included in most commercially available test strips, and are applicable to animal specimens.

For routine toxicology studies, either semi-quantitative or quantitative measurements are acceptable as standard practice. However, quantitative evaluation of protein, glucose, electrolytes, creatinine, biomarkers of renal injury, and/or fractional electrolyte excretion (if concurrent serum chemistry data are available) should be considered when these data can provide critical information on efficacy or risk assessment. ASVCP RAC recommends that quantitative urinalysis results be normalized to urine creatinine concentration and/or for urine volume for accurate interpretation of changes.⁵⁹

Nonroutine variables and analytes

The utilization of noninvasive biomarkers to evaluate various systems allows for enhanced compound screening and dose selection, and is recognized as an important supplementary approach to developing novel drugs with an optimal safety profile, but is not subject to specific regulatory guidances. Recent advances in technology have improved the throughput and sensitivity of biomarker assays. For example, troponins and acute phase proteins such as C-reactive protein (CRP) can be easily measured due to the development and availability of ultrasensitive immunoassay systems.^{60–62} Multiplex assays (such as bead-based assays utilizing Luminex [Minneapolis, MN, USA] technology) are commonly utilized for the measurement of cytokines, while immunochemistry can identify hormones and functional proteins, and high-performance liquid chromatography can be utilized to analyze trace elements. Genomics, proteomics, and metabonomics approaches have also been used successfully to screen for useful biomarkers of toxicity. A comprehensive understanding of the limi-

tations of available methodologies is essential to ensure that the most appropriate methods are used for analyses.⁶³

Cytologic specimens

Regulatory agencies do not provide specific guidances for the collection and evaluation of cytologic specimens, with the exception of bone marrow cytology. However, cytologic evaluations are used in nonregulated and regulated studies to help characterize test article-related effects.

Broncho-alveolar lavage

Broncho-alveolar lavage fluid (BALF) collection is performed to evaluate cells present in the lower airways. It can be used to evaluate the development and/or resolution of primary or secondary lung changes in animals exposed to test articles,^{64,65} and in some instances, can aid in the detection of early effects within the lung, which precede histologic findings. This method may also aid in the determination of dose–response curves for inhaled test articles.

Broncho-alveolar lavage fluid is collected under anesthesia⁶⁶ or at necropsy. The lungs are infused with saline through a transtracheal catheter or directly instilled at necropsy. The infused fluid is then re-aspirated and collected. At necropsy, massage of the lungs prior to re-aspiration of the fluid is not recommended, as this may cause variability between the samples. Standardization of saline instillation methods is necessary to obtain reproducible results.

Recommended BALF analysis includes total nucleated cell count (TNCC) and differential cell count. The differential cell count is considered essential for the interpretation and is not dependent on the volume of fluid collected. The TNCC values should be interpreted with caution as the number of cells present in the sample depends on fluid volumes infused and collected. Biochemistry and cytokine analysis can also be measured on BALF: total protein and CRP concentrations are considered useful markers of inflammation,⁶⁵ and lactate dehydrogenase (LDH) activity is a marker of cell damage.^{67–69}

Standardized analytical methods should be used throughout, and between studies for the same test article. A common practice is to pool all fluids from different instillations of a single animal at a single sampling period, perform TNCC and differential counts, centrifuge the remaining fluid, and collect the supernatant for biochemistry analysis. The utility of collecting BALF in EDTA-coated tubes has been challenged as

BALF cells do not clump easily and clump less when the lungs are not massaged.⁷⁰ However, it is the authors' opinion that cells are better preserved in EDTA (K2 or K3EDTA). The ASVCP RAC recommendation is to collect BALF from the first instillation in tubes without anticoagulant for biochemistry or cytokine analysis, and from subsequent instillations into EDTA-coated tubes for TNCC and differential counts.

ASVCP RAC recommends manual 200-cell differential counts of appropriately stained (eg, Wright–Giemsa) slides prepared by cytocentrifugation. Hematology analyzers equipped with multispecies software can be used for automated counts and validated to perform TNCCs.^{71,72} However, most automated hematology analyzers do not adequately differentiate various BALF cell types; multispecies software developed for BALF analysis appears to offer promising results.^{73,74} The reader is referred to a cytology textbook for the interpretation of the different cell types.⁷⁵

Other body fluids (cerebrospinal fluid, synovial, pleural, and abdominal fluids)

Analysis of body fluids during toxicity studies, such as cerebrospinal fluid (CSF), synovial, pleural, and abdominal fluids, is performed in a similar manner to diagnostic laboratories. Fluids should be collected directly in EDTA (K2 EDTA is preferred if the collection volume is low) tubes and refrigerated if not analyzed immediately. However, cells tend to degrade rapidly and most fluids should be analyzed within a few hours of sample collection.^{76,77} Manual counts of nucleated cells are performed using a hemocytometer. Cellular differential counts are performed on appropriately stained (eg, Wright–Giemsa) direct smears or cytocentrifuge preparations. Cytocentrifuge preparations may not provide an adequate repartition of synovial cells, and therefore, direct smears prepared immediately following collection are preferable for cell differential counts. Cytocentrifugation of body fluid samples may not be required when cellularity is increased. CSF generally contains extremely low protein concentrations; therefore, an ultrasensitive urine protein assay should be used.⁷⁸ Total proteins may be measured on automated chemistry analyzers or by refractometry for all other fluids.

Bone marrow cytology

Regulatory agencies do not mandate the evaluation of bone marrow cytology, but FDA recommends slide preparation in toxicity studies for evaluation if unexplained hematologic effects are identified.⁴¹ ASVCP RAC recommends the collection of bone marrow

smears from all regulated studies (carcinogenicity studies should be considered on a case-by-case basis⁴³) for possible future evaluation, if indicated. Best practices for bone marrow evaluation, including sample collection, evaluation methods, and reporting results in the context of other relevant data, are included in a recent paper produced by the Bone Marrow Working Group of ASVCP/STP.⁷⁹ ASVCP RAC supports these thorough and recent best practices.

Data interpretation and reporting

Data interpretation

Many aspects require consideration in the interpretation of study-related clinical pathology data and a single method of data interpretation cannot cover all potential scenarios. Instead, a qualified individual (refer to Veterinary Clinical Pathologist) should evaluate the data from each study on a case-by-case basis using a subjective “weight-of-evidence” approach. This section provides a review of general guidances for interpreting clinical pathology variables and analytes, and reporting the pathology interpretation in nonclinical studies.^{49,80}

For all nonclinical test species, the relationship of a change in any given variable or analyte to test article administration and/or experimental conditions and determination of its relevance to human risk assessment requires comparison of individual and group mean or median data with that of concurrent controls. For nonrodent (such as dogs, NHP, and minipigs) studies, interpretation of the change should also include comparisons with pretreatment values on an individual animal basis.⁸¹ Comparison with pretreatment values is also crucial for the interpretation of novel variables and analytes (ie, new biomarkers) in all species. In addition, interpretation of the pattern of changes rather than the qualitative or quantitative change of an individual variable or analyte improves meaningful data interpretation and understanding of the relevance of findings. Finally, clinical pathology variables and analytes should be evaluated in the context of other available study data because determination of test article-related and adverse effects requires the integrated assessment of clinical pathology data with other pathology findings, in-life observations, exposure and metabolism data, and, when known, anticipated pharmacological effects of the test article.

Factors for consideration in deciding whether a change is test article-related include the magnitude and incidence of the change, dependency of the

response on dose or duration, correlative changes in in-life or other pathology data, consistency of the change with known target-mediated responses, potential range of individual variability, and effects of study-related procedures such as blood collection or anesthesia.⁸²

Clinical pathology data interpretation of test article-related effects (adverse and nonadverse) should be used to help determine the no-observed-effect-level (NOEL) or the more routinely used, no-observed-adverse-effect-level (NOAEL).⁸³

Criteria that can be considered to differentiate adverse from nonadverse test article-related findings include, but are not limited to, adverse impact on function or life of the animal, magnitude, and direction of the changes, potential adverse impact on human patients, and monitorability in nonclinical species and human patients.⁸⁴

There are no standard statistical analyses that can be used for all clinical pathology data. Rather, the statistical analysis of the data is dictated by the type, variability, and distribution of the data. In data interpretation, statistics are a useful aid to identify mathematical differences between control and test article-treated groups or to characterize a trend in the data that may be related to dose and/or duration. As such, statistics supplement rather than replace the process of data interpretation. Individual animal variability and the low number of animals in nonrodent animal studies complicate the statistical analysis of the data.^{40,82} Importantly, not all statistically significant changes are test article related and not all test article-related changes are statistically significant. For a review of statistics in toxicology studies, including clinical pathology data, the reader is directed to pertinent references on this subject.^{85,86}

Data reporting

Best practices for reporting the interpretation of pathology data, including clinical pathology data from GLP studies, have been published to “guarantee the quality and reliability of pathology data and interpretations incorporated within animal toxicology studies”⁸⁰ and are summarized below. Importantly, these recommendations are also applicable to non-GLP and discovery studies.

All clinical pathology data generated or evaluated for the study should be presented in individual animal and summary data tables. Statistically significant findings should be clearly identified in the tables. Tabular or graphic data can be embedded, when appropriate, into the narrative to bring impactful findings to the attention of the reader.

The report text should describe test article-related clinical pathology findings, correlate them with study-related data, and address their toxicologic or biologic significance. When applicable, the report should also address why some apparent changes are not considered test article related or findings are not toxicologically relevant, characterize pharmacologically mediated findings, and include supportive literature references. Finally, the report text should summarize the test article-related findings and the doses (exposures) at which these findings occurred.

Clinical pathology findings can be presented within an integrated pathology report containing the graphical or tabular data and narrative or as a stand-alone contributor report. In either case, the interpretation of the data must be accompanied by the signature of the responsible scientist to identify their contributions, and to reflect agreement with the presentation of the findings and responsibility for the sample analysis and data generation (in conjunction with the laboratory) and data interpretation and reporting.⁸⁰ With either a stand-alone or integrated pathology report, the authenticity of the pathology data interpretation must be maintained.

Peer review

The peer review provides a consensus on the integrated interpretation and summary of the clinical pathology data. Although recent publications have generally focused on the peer review of morphologic pathology data,^{87,88} these principles are also applicable to the peer review of clinical pathology data. A peer review of either dataset is considered separate from management review. The qualified peer reviewer (refer to Veterinary Clinical Pathologist) for the clinical pathology data is responsible for ensuring that the study pathologist's or study scientist's interpretation, report, and integration with other study data are complete and with consensus. ASVCP RAC considers the informal peer review (ie, no formal signed documentation by the peer-review pathologist) of clinical pathology data interpretation a best practice. Documentation of this process is not necessary as clinical pathology data and tables are electronically generated by automated analyzers, traced, formatted, and archived. In contrast, morphologic pathology data (ie, the data tables) are generated by a subjective process of interpretation and refinement by a study pathologist, and generally in regulated toxicity studies, with the additional review of a qualified peer reviewer.

Reference intervals

The process for characterization of a reference interval (RI, also called reference range) can be extensive and,

as such, is beyond the scope of this paper. The reader is, therefore, referred to recent and comprehensive reviews on this topic.^{89–91}

The comparison of a change in a variable with the limits or range of the RI should not be used to identify test article-related or adverse effects, but rather as additional information to support interpretation. Specifically, values that fall outside the range of the reference interval cannot be assumed to be test article related or adverse findings. Likewise, values that fall within the interval should not automatically be considered "normal," unrelated to the test article, or of no impact to the human risk assessment.^{82,92} However, RIs and/or historical control data can be useful tools for providing a context for assessing whether a finding is adverse or if there are aberrant values within the control group.

Veterinary clinical pathologist and laboratory personnel

Veterinary clinical pathologist

Veterinary clinical pathologists are individuals with a doctorate in veterinary medicine plus formal training in veterinary clinical pathology, and are also generally expected to obtain board certification in veterinary clinical pathology (American College of Veterinary Pathologists, <http://www.acvp.org/residents/Exam/Scope.cfm> [accessed June 25, 2013] or European College of Veterinary Clinical Pathology, <http://www.ecvcp.org> [accessed June 25, 2013]). At minimum, veterinary clinical pathologists are formally trained in hematology, clinical biochemistry, urinalysis, cytology, and surgical pathology, and have in-depth knowledge of these diagnostic modalities. In Europe, training also includes laboratory management. Furthermore, a board-certified individual is proficient at recognizing and interpreting clinical pathology datasets and has a thorough understanding of pathogenic mechanisms of disease, species differences, testing methods and validation, and instrumentation.

Regulations issued by FDA, 21 CFR, Part 58, Good Laboratory Practice Regulations¹ state that "Each individual engaged in the conduct of or responsible for the supervision of a nonclinical laboratory study shall have the education, training, and experience, or combination thereof, to enable the individual to perform the assigned functions." In agreement with these regulations, toxicologic pathologist-derived best practices concur that a study pathologist or scientist analyzing data or reporting clinical pathology findings for GLP toxicology studies "must have the education, qualifica-

tions and experience to perform these tasks and to integrate pathology with clinical signs, exposure information and other study information.⁸⁰ Having a qualified clinical pathologist analyze and interpret clinical pathology data for discovery and toxicology studies can help ensure that the clinical pathology report accurately reflects the raw data in a clear, concise, logical, and accurate manner.^{93,94} This facilitates understanding of data significance by regulatory reviewers,⁹⁵ correlation of clinical pathology results with other study findings, and translation of data to the clinical/human setting. Because toxicity is a leading cause of attrition during drug development, the veterinary clinical pathologist can play a crucial role in the understanding of toxicity evident from clinical pathology data.^{93,96,97} Clinical pathologists currently and capably perform many other roles and responsibilities in industry, including (1) clinical pathology laboratory management, (2) clinical pathology test or biomarker development, validation, and implementation, (3) clinical pathology peer review, (4) bone marrow and cytology evaluation and reporting, (5) clinical veterinary diagnostic efforts, (6) in-house clinical pathology consultation for discovery, translational, and/or clinical activities, (7) contributions to the integrated toxicology reports and regulatory documents, such as investigational new drug applications or new drug applications, regulatory responses, in-licensing decisions, and the clinical drug development plan, and (8) drug safety leader for project teams.

Employing individuals with a doctorate of veterinary medicine or equivalent, a relevant advanced science degree (master of science or doctorate of philosophy), formal training in veterinary clinical pathology, and board certification by the American College of Veterinary Pathologists or the European College of Veterinary Clinical Pathologists is considered a best practice. While there are clinical pathologists without advanced degrees or board certification, the combination of credentials described provides a skill set that is mutually beneficial to the employer and the clinical pathologist. These ASVCP RAC recommendations regarding roles and responsibilities of veterinary clinical pathologists in the biopharmaceutical industry are consistent with the previously published literature and predicted trends on this topic.^{94,97}

Expanded roles for veterinary clinical pathologists

Veterinary clinical pathologists provide expertise throughout the nonclinical drug development process⁹³ but also have capabilities, highlighted below, in the clinical and research arenas.

Translational Research and Medicine. Translational research applies knowledge from the basic sciences to clinical settings and can be referred to as “bench to bedside” research (<https://www.iths.org/>). Veterinary clinical pathologists can play a critical role in characterization of animal models and their relevance to the clinic or assessment of translation from discovery and efficacy studies to human or veterinary clinical trials.

Biomarker selection and use allow more effective decision making from discovery nonclinical to clinical studies and is at the core of many successful Proof-of-Concept (POC) clinical studies. The veterinary clinical pathologist is adept at assisting with development of nonclinical assays and interpretation, integration, and “translation” of results to clinical trials.

Clinical Pathology in Clinical Development. Clinical pathology is often a key monitoring tool linking clinical signs to developing disease and is used routinely for safety assessment in both nonclinical and clinical (human and veterinary) studies.

The veterinary clinical pathologist can also provide expertise regarding the conduct, interpretation, and reporting of human and veterinary clinical trials as principal investigators in clinical trials (eg, writing clinical study protocols and reports), clinical scientists for product development with a study sponsor (eg, writing investigator’s brochures), and review members for institutional review boards (eg, evaluating clinical study protocols), and during pharmacovigilance (eg, evaluating adverse events in clinical trials).

Laboratory professional staff

The regulatory requirements for technical staff working on GLP studies, which includes individuals working in the clinical pathology laboratory, indicate that all individuals engaged in the conduct of a nonclinical laboratory study “shall have the education, training and experience, or combination thereof, to perform the assigned functions.”

ASVCP RAC recommends that laboratory personnel meet the qualifications for a medical technologist (BS or equivalent), clinical laboratory scientist (BS or equivalent), or at the minimum, a medical technician (AS or equivalent). This recommendation is based on the training acquired by these individuals in the areas of clinical pathology such as biomedical instrumentation, hematology, coagulation, clinical chemistry, and immunology. The integrity of the data produced in the clinical pathology laboratory is not only dependent on the input of the clinical pathologist but depends heavily on the ability of the laboratory staff to maintain the integrity of the sample, assay, and instrument function, and thus minimize preanalytical, analytical,

and postanalytical error. Certified medical technologists and laboratory scientists specifically trained to collect and analyze biologic samples from people should also receive documented training in the analysis of nonclinical veterinary clinical pathology samples.

Conclusions

As veterinary clinical pathologists have become more routinely involved in the pharmaceutical and biotechnology industries, an evolution of laboratory practices has occurred. For example, there is an improved understanding of the impact of parameters such as blood collection site, urine collection methods, species-specific test selection, and instrumentation on the results generated. Based on the collective experience of the clinical pathologists in the ASVCP RAC, data interpretation, as a whole, has become increasingly integrative, thus improving the understanding of the relevance of findings to toxicity and human risk assessment.

The principles of best practice in veterinary toxicologic clinical pathology allow parallel operations and practice with comparable standard operating procedures for both GLP and non-GLP laboratories. A “fit-for-purpose” approach to validation and documentation allows the rigor of validation and documentation to fit the needs of the study, and may differ between discovery and exploratory non-GLP assays and those for regulated GLP studies.

In conclusion, while veterinary clinical pathology practices are similar across the industry, common practices are not always considered “best practice.” In this paper, the ASVCP RAC reviewed the current regulatory guidances and literature, provided clarity on areas of clinical pathology practice that are not aligned, and provided the ASVCP RAC consensus on best practices for clinical pathology in the pharmaceutical and biotechnology industries. These best practices consolidate information from many areas to provide a tool for those involved in clinical pathology data generation and interpretation in industry.

Acknowledgments

We acknowledge the scientific and technical contributions of Deborah Wescott, Bristol-Myers Squibb Company. The Scientific and Regulatory Policy Committee (SRPC) of STP, particularly Drs. Michael Peden and Marielle Odin, and the clinical and toxicologic pathology scientific communities provided highly beneficial and much appreciated detailed review of this manuscript.

References

1. Food and Drug Administration (FDA). Good Laboratory Practice for nonclinical laboratory studies, 21 CFR Part 58, 2012 Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?CFRPart=58>. Accessed June 20, 2013.
2. European Medicines Agency (EMA). Note for Guidance of Good Clinical Practice. CPMP/ICH, 2002 Available at: http://www.edctp.org/fileadmin/documents/EMA_ICH-GCP_Guidelines_July_2002.pdf. Accessed June 25, 2013.
3. Organisation for Economic Co-operation and Development (OECD). OECD series on principles of good laboratory practice and compliance monitoring: Environment Directorate, Organisation for Economic Co-operation and Development, 1998 Available at: [http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem\(98\)17&doclanguage=en](http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en). Accessed June 25, 2013.
4. Organisation For Economic Co-Operation And Development (OECD). OECD series on principles of good laboratory practice and compliance monitoring: The application of the principles of GLP to computerised systems, 1995 Available at: [http://search.oecd.org/officialdocuments/displaydocumentpdf/?doclanguage=en&cote=ocde/gd\(95\)115](http://search.oecd.org/officialdocuments/displaydocumentpdf/?doclanguage=en&cote=ocde/gd(95)115). Accessed 25 June 2013.
5. Food and Drug Administration (FDA). Food and Drugs, Title 21, Part 11 - Electronic Records, Electronic Signatures, 2012 Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=11&showFR=1>. Accessed 25 June 2013.
6. Flatland B, Freeman KP, Friedrichs KR, et al. ASVCP quality assurance guidelines: control of general analytical factors in veterinary laboratories. *Vet Clin Pathol*. 2010;39:264–277.
7. Gunn-Christie RG, Flatland B, Friedrichs KR, et al. ASVCP quality assurance guidelines: control of preanalytical, analytical, and postanalytical factors for urinalysis, cytology, and clinical chemistry in veterinary laboratories. *Vet Clin Pathol*. 2012;41:18–26.
8. Vap LM, Harr KE, Arnold JE, et al. ASVCP quality assurance guidelines: control of preanalytical and analytical factors for hematology for mammalian and nonmammalian species, hemostasis, and cross-matching in veterinary laboratories. *Vet Clin Pathol*. 2012;41:8–17.
9. ASVCP. Newly formatted and revised ASVCP Quality Control Guidelines. Principles of Quality Assurance and Standards for Veterinary Clinical Pathology, 2009 Available at: <http://www.asvcp.org/pubs/pdf/ASVCPQualityControlGuidelines.pdf>. Accessed 25 June 2013.

10. Lee JW, Devanarayan V, Barrett YC, et al. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res*. 2006;23:312–328.
11. Shah VP, Midha KK, Findlay JWA, et al. Bioanalytical method validation-A revisit with a decade of progress. *Pharm Res*. 2000;17:1551–1557.
12. Shah VP. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. *AAPS J*. 2007;9:E43–E47.
13. Viswanathan CT, Bansal S, Booth B, et al. Workshop/conference report-quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J*. 2007;9:E30–E42.
14. Miller KJ, Bowsher RR, Celniker A, et al. Workshop on bioanalytical methods validation for macromolecules: summary report. *Pharm Res*. 2001;18:1373–1383.
15. Smolec J, DeSilva BS, Smith W, et al. Bioanalytical method validation for macromolecules in support of pharmacokinetic studies. *Pharm Res*. 2005;22:1425–1431.
16. Food and Drug Administration (FDA). Guidance for Industry: Bioanalytical Method Validation: 2001. Available at: <http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf>. Accessed 24 June 2013.
17. European Medicines Agency (EMA). Guideline on bioanalytical method validation. 2011. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf. Accessed 24 June 2013.
18. Jensen AL, Kjølgaard-Hansen M. Method comparison in the clinical laboratory. *Vet Clin Pathol*. 2006;35:276–286.
19. Lee JW, Weiner R, Bowsher RR, et al. Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development: a conference report. *Pharm Res*. 2005;22:499–511.
20. Lee JW, Pan P, O'Brien P, et al. Development and validation of ligand-binding assays for biomarkers. In: Khan MN, Findlay JWA, eds. *Ligand-binding Assays: development, Validation, and Implementation in the Drug Development Arena*. Hoboken, NJ: John Wiley and Sons, Inc.; 2010;129–162.
21. DeSilva BS, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm Res*. 2003;20:1885–1990.
22. Findlay JWA, Smith WC, Lee JW, et al. Validation of immunoassays for bioanalysis: a pharmaceutical perspective. *J Pharmaceut Biomed Anal*. 2000;21:1249–1273.
23. Bansal S, DeStefano A. Key elements of bioanalytical method validation for small molecules. *AAPS J*. 2007;9:E109–E114.
24. Findlay JWA, Dillard RF. Appropriate calibration curve fitting in ligand binding assays. *AAPS J*. 2007;9:E260–E267.
25. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of analytical procedures, Q2(R1). 1995. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf. Accessed 24 June 2013.
26. Fitzner Toft M, Petersen MH, Dragsted N, Hansen AK. The impact of different blood sampling methods on laboratory rats under different types of anaesthesia. *Lab Anim*. 2006;40:261–274.
27. Evans GO. *Animal Clinical Chemistry: a practical guide for toxicologists and biomedical researchers*. 2nd ed. Boca Raton, FL: CRC Press; 2009.
28. Diehl KH, Hull R, Morton D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol*. 2001;21:15–23.
29. Eory ML, Zanuzzi CN, Fuentealba NA, et al. Effects of different anesthetics in the murine model of EHV-1 infection. *Vet Pathol* 2013; published Feb 4, 2013 online before print.
30. Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. *Lab Anim (NY)*. 2005;34:39–43.
31. International Council for Standardization in Haematology. Recommendations of the International Council for standardization in haematology for ethylenediaminetetraacetic acid anticoagulation of blood for blood cell counting and sizing. International Council for standardization in haematology: expert panel on cytometry. *Am J Clin Pathol* 1993;100:371–372.
32. National Committee for Clinical Laboratory Standards. *Document H1-A5 Tubes and additives for venous blood specimen collection; approved standard*. 5th ed. 2003. Available at: <http://isoforlab.com/phocadownload/csli/H1-A5.pdf>. Accessed June 25, 2013.
33. Furlanello T, Caldin M, Stocco A, et al. Stability of stored canine plasma for hemostasis testing. *Vet Clin Pathol*. 2006;35:204–207.
34. Ameri M, Schnaars HA, Sibley JR, Honor DJ. Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4 degrees C in EDTA using the ADVIA 120 hematology analyzer. *Vet Clin Pathol*. 2011;40:188–193.

35. Cora MC, King D, Betz LJ, Wilson R, Travlos GS. Artifactual changes in sprague–dawley rat hematologic parameters after storage of samples at 3 °C and 21 °C. *J Am Assoc Lab Anim Sci.* 2012;51:616–621.
36. Weiser G. Laboratory technology for veterinary medicine. In: Thrall M, ed. *Veterinary Hematology and Clinical Chemistry*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2012.
37. Kurata M, Horii I. Blood coagulation tests in toxicological studies—review of methods and their significance for drug safety assessment. *J Toxicol Sci.* 2004;29:13–32.
38. Adcock D, Kressin D, Marlar RA. The effect of time and temperature variables on routine coagulation tests. *Blood Coagul Fibrinolysis.* 1998;9:463–470.
39. Stockham S, Scott M. *Fundamentals of Veterinary Clinical Pathology*. Ames, Iowa: Blackwell Publishing; 2008.
40. Weingand K, Brown G, Hall R, et al. Harmonization of animal clinical pathology testing in toxicity and safety studies. The Joint Scientific Committee for International Harmonization of Clinical Pathology Testing. *Fundam App Toxicol.* 1996;29:198–201.
41. Food and Drug Administration. Guidance for Industry and Other Stakeholders. Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000: IV.B.1 General Guidelines for Designing and Conducting Toxicity Studies: Center for Food Safety and Applied Nutrition, 2003. Available at: <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf>. Accessed June 25, 2013.
42. Organisation for Economic Co-operation and Development (OECD). OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Publications de l'OCDE, 2009.
43. Young JK, Hall RL, O'Brien P, Strauss V, Vahle JL. Best practices for clinical pathology testing in carcinogenicity studies. *Toxicol Pathol.* 2011;39:429–434.
44. European Agency for the Evaluation of Medicinal Products Committee for Proprietary Medicinal Products. Note for Guidance on Carcinogenic Potential, 2002 (CPMP/SWP/2877/00).
45. Food and Drug Administration. Guidance for Industry and Other Stakeholders. Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000: IV.C.6 Carcinogenicity Studies with Rodents, Center for Food Safety and Applied Nutrition, 2006. Available at: <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf>. Accessed June 25, 2013.
46. Etzell JE. For WBC differentials, report in absolute numbers. College of American Pathologists (CAP) Today Newsletter, March 2010. Available at: <http://www.cap.org>. Accessed June 24, 2013.
47. Schultze AE, Walker DB, Turk JR, Tarrant JM, Brooks MB, Pettit SD. Current practices in preclinical drug development: gaps in hemostasis testing to assess risk of thromboembolic injury. *Toxicol Pathol.* 2013;41:445–453.
48. Environmental Protection Agency (EPA). OPPTS 870.3050 Repeated Dose 28-Day Oral Toxicity Study in Rodents, Health Effects Test Guidelines, 2000.
49. Boone L, Meyer D, Cusick P, et al. Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. *Vet Clin Pathol.* 2005;34:182–188.
50. Boyd JW. The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Vet Clin Pathol.* 1983;12:9–24.
51. Boyd JW. Serum enzymes in the diagnosis of disease in man and animals. *J Comp Pathol.* 1988;98:381–404.
52. Valentine BA, Blue JT, Shelley SM, Cooper BJ. Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog. *J Vet Intern Med.* 1990;4:140–143.
53. Evans GO, Parsons CE. Potential errors in the measurement of total protein in male rat urine using test strips. *Lab Anim.* 1986;20:27–31.
54. Klausner JS, Osborne CA, Stevens JB. Clinical evaluation of commercial reagent strips for detection of significant bacteriuria in dogs and cats. *Am J Vet Res.* 1976;37:719–722.
55. Taylor DM, Neal DL. False negative hyperglucosuria test-strip reactions in laboratory mice. *Lab Anim.* 1982;16:192–197.
56. Allchin JP, Evans GO. A comparison of three methods for determining the concentration of rat urine. *Comp Biochem Physiol A Comp Physiol.* 1986;85:771–773.
57. Vail DM, Allen TA, Weiser G. Applicability of leukocyte esterase test strip in detection of canine pyuria. *J Am Vet Med Assoc.* 1986;189:1451–1453.
58. Paquignon A, Tran G, Provost JP. Evaluation of the Clinitek 200 urinary test-strip reader in the analysis of dog and rat urines in pre-clinical toxicology studies. *Lab Anim.* 1993;27:240–246.
59. Goldstein SL. Urinary kidney injury biomarkers and urine creatinine normalization: a false premise or not? *Kidney Int.* 2010;78:433–435.
60. Schultze AE, Main BW, Hall DG, et al. A comparison of mortality and cardiac biomarker response between three outbred stocks of Sprague Dawley rats treated with isoproterenol. *Toxicol Pathol.* 2011;39:576–588.

61. Apple FS, Murakami MM, Ler R, Walker D, York M. Analytical characteristics of commercial cardiac troponin I and T immunoassays in serum from rats, dogs, and monkeys with induced acute myocardial injury. *Clin Chem* 2008;54:1982–1989.
62. Watterson C, Lanevski A, Horner J, Loudon C. A comparative analysis of acute-phase proteins as inflammatory biomarkers in preclinical toxicology studies: implications for preclinical to clinical translation. *Toxicol Pathol.* 2009;37:28–33.
63. Allinson J, Brooks S. Biomarkers in drug development—a CRO perspective. *Curr Sep.* 2004;21:15–19.
64. Dorato MA. Overview of inhalation toxicology. *Environ Health Perspect.* 1990;85:163–170.
65. Henderson RF. Use of bronchoalveolar lavage to detect respiratory tract toxicity of inhaled material. *Exp Toxicol Pathol.* 2005;57(Suppl. 1):155–159.
66. Porter DW, Hubbs AF, Baron PA, et al. Pulmonary toxicity of Expancel microspheres in the rat. *Toxicol Pathol.* 2007;35:702–714.
67. Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. *Eur Respir J.* 1996;9:1736–1742.
68. Beck BD, Gerson B, Feldman HA, Brain JD. Lactate dehydrogenase isoenzymes in hamster lung lavage fluid after lung injury. *Toxicol Appl Pharmacol.* 1983;71:59–71.
69. DeNicola DB, Rebar AH, Henderson RF. Early damage indicators in the lung. V. Biochemical and cytological response to NO₂ inhalation. *Toxicol Appl Pharmacol.* 1981;60:301–312.
70. Ma-Hock L, Burkhardt S, Strauss V, et al. Development of a short-term inhalation test in the rat using nanotitanium dioxide as a model substance. *Inhal Toxicol.* 2009;21:102–118.
71. Harris N, Kunicka J, Kratz A. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. *Lab Hematol.* 2005;11:47–61.
72. Hatzelmann A, Haefner D, Beume R, Schudt C. Automatic leukocyte differentiation in bronchoalveolar lavage fluids of guinea pigs and brown-Norway rats. *J Pharmacol Toxicol Methods.* 1996;35:91–99.
73. Natiello M, George K, Lamca J, Zelmanovic D, Chapman RW, Phillips JE. Manual and automated leukocyte differentiation in bronchoalveolar lavage fluids from rodent models of pulmonary inflammation. *Comp Clin Pathol.* 2009;18:101–111.
74. Mathers R, Evans G, Bleby J, Tornow T. Total and differential leucocyte counts in rat and mouse bronchoalveolar lavage fluids using the sysmex XT-2000iV. *Comp Clin Pathol.* 2007;16:29–39.
75. Cowell RL, Tyler RD, Meinkoth JH, De Nicola DB. *Diagnostic Cytology and Hematology of the Dog and Cat.* 3rd ed. St. Louis, MO: Mosby; 2008.
76. Sharkey LC, Dial SM, Matz ME. Maximizing the diagnostic value of cytology in small animal practice. *Vet Clin North Am Small Anim Pract.* 2007;37:351–372, vii.
77. Bohn AA, Callan RJ. Cytology in food animal practice. *Vet Clin North Am Food Anim Pract* 2007;23:443–479, vi.
78. Raskin RE, Meyer DJ. The Central Nervous System, Cerebrospinal fluid. In: Elsevier, ed. *Canine and Feline Cytology- A Color Atlas and Interpretation Guide.* 2nd ed, St. Louis, MO: Saunders; 2009:325–346.
79. Reagan WJ, Irizarry-Rovira A, Poitout-Belissent F, et al. Best practices for evaluation of bone marrow in nonclinical toxicity studies. *Vet Clin Pathol.* 2011;40:119–134.
80. Morton D, Kemp RK, Francke-Carroll S, et al. Best practices for reporting pathology interpretations within GLP toxicology studies. *Toxicol Pathol.* 2006;34:806–809.
81. Hall RL, Everds NE. Factors affecting the interpretation of canine and nonhuman primate clinical pathology. *Toxicol Pathol.* 2003;31(Suppl.):6–10.
82. Hall RL, Everds NE. Principles of clinical pathology for toxicology studies. In: Hayes AW, ed. *Principles and Methods of Toxicology.* Boca Raton, FL: Taylor and Francis, LLC, 2008;1317–1358.
83. Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F. Recognition of adverse and nonadverse effects in toxicity studies. *Toxicol Pathol.* 2002;30:66–74.
84. European Center of Ecotoxicology and Toxicology of Chemicals (ECETOC). Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies—Technical report no. 85. 2002. Available at: <http://members.ecetoc.org/Documents/Document/TR%20085.pdf>. Accessed June 24, 2013.
85. Gad SC. Statistics for toxicologists. In: Hayes AW, ed. *Principles and Methods of Toxicology.* Boca Raton, FL: Taylor and Francis, 2008;369–452.
86. Waner T. Current statistical approaches to clinical pathology data from toxicological studies. *Toxicol Pathol.* 1992;20:477–479.
87. McKay JS, Barale-Thomas E, Bolon B, et al. A commentary on the process of peer review and pathology data locking. *Toxicol Pathol.* 2010;38:508–510.

88. Morton D, Sellers RA, Barale-Thomas E, et al. Recommendations for pathology peer review. *Toxicol Pathol.* 2010;38:118–1127.
89. Geffré A, Friedrichs K, Harr K, Concordet D, Trumel C, Braun JP. Reference values: a review. *Vet Clin Pathol.* 2009;38:288–298.
90. Friedrichs KR, Harr KE, Freeman KP, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol.* 2012;41:441–453.
91. Walton RM. Subject-based reference values: biological variation, individuality, and reference change values. *Vet Clin Pathol.* 2012;41:175–181.
92. Hall RL. Lies, damn lies, and reference intervals (or hysterical control values for clinical pathology data). *Toxicol Pathol* 1997;25:647–649; discussion 650–641.
93. Schultze AE, Sullivan JM. Screening a novel class of anticoagulants for multi-organ toxicity using Fischer 344 rats: involvement of clinical and anatomic pathologists in the early drug development process. *Vet Clin Pathol.* 2007;36:123–136.
94. Schultze AE, Bounous DI, Bolliger AP. Veterinary clinical pathologists in the biopharmaceutical industry. *Vet Clin Pathol.* 2008;37:146–158.
95. Black HE. Design and writing of the preclinical safety report. *Toxicol Pathol.* 1994;22:202–205.
96. Kramer JA, Sagartz JE, Morris DL. The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. *Nat Rev Drug Discov.* 2007;6:636–649.
97. Hall RL. Veterinary clinical pathology in industry: 2000 to 2020. *Vet Clin Pathol.* 2000;29:75–76.