Chapter 7. Clinical Pathology

Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences

From: Roberts GK, Stout MD, editors. Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences. Research Triangle Park, NC: National Institute of Environmental Health Sciences; 2023.
https://doi.org/10.22427/NIEHS-00

7. Clinical Pathology

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Last Updated: March 2023

At a minimum, the laboratory must be capable of satisfactorily performing the clinical pathology assessments/measurements listed in this chapter.

7.1. Hematology

The laboratory must be capable of performing the following required hematology measurements using automated or semi-automated systems (impedance or laser-optic instruments) optimized and validated for rodent species:

- Erythrocyte count
- Hemoglobin concentration
- Hematocrit (automated)
- Mean corpuscular volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration
- Leukocyte count
- Leukocyte differential count
- Reticulocyte count
- Platelet count
- Cell hemoglobin concentration mean (CHCM)
- Cellular hemoglobin (CH)

If the laboratory lacks a hematology analyzer that measures CHCM and CH, a spun (manual method) hematocrit (packed cell volume) shall be performed.

Blood smears shall be made for each animal. A morphological assessment (microscopic evaluation) of erythrocytes, leukocytes, and platelets shall be performed and documented. Nucleated erythrocyte (nRBC) counts (nRBC/100 leukocytes) shall be reported. The requirement for blood smear evaluation and nRBC counts may be waived **ONLY** if the laboratory has approved blood smear review guidelines in an applicable hematology standard operating procedure (SOP).

Instead of an automated leukocyte differential count, a leukocyte differential count—determined by microscopic examination of a Wright's-type stained blood smear and identification of at least 100 leukocytes (manual method)—is acceptable. A manual differential count will be performed if the automated leukocyte count or leukocyte differential count generates instrument errors or

abnormal cell counts/distributions/findings—or as outlined in an approved applicable hematology SOP.

Laboratories shall possess the capability to perform an automated reticulocyte count. If an automated reticulocyte count cannot be performed, a reticulocyte count determined by microscopic examination (manual method) of a supravitally stained blood smear (e.g., new methylene blue) is acceptable. The manual reticulocyte count must be reported as an absolute number based on the proportion of reticulocytes in 1,000 erythrocytes or by use of a Miller disc.

Platelet, reticulocyte, and leukocyte cell counts will be expressed as absolute counts. The raw data will be determined by electronic or laser-optic methods.

When a manual leukocyte differential count is required (see above), absolute leukocyte cell counts may be derived by calculation using the instrument-derived total leukocyte count and the microscopically derived percentages obtained for the individual cell types. The reporting of data based on percentages, estimates, and manual (i.e., hemocytometer) counts is not acceptable.

7.2. Clinical Chemistry

The laboratory must be capable of performing the following required serum clinical chemistry measurements using automated or semi-automated systems optimized and validated for rodent species:

- Total protein concentration
- Albumin concentration
- Globulin concentration (total protein minus albumin)
- Albumin/globulin ratio
- Urea nitrogen concentration
- Creatinine concentration
- Alanine aminotransferase activity
- Sorbitol dehydrogenase activity
- Alkaline phosphatase activity
- Total bile acid concentration
- Aspartate aminotransferase activity
- Total bilirubin concentration
- Direct bilirubin concentration
- Indirect bilirubin concentration (total bilirubin minus direct bilirubin)
- Glucose concentration
- Creatine kinase activity
- Cholesterol concentration
- Triglyceride concentration

7.3. Urinalysis

The laboratory must be capable of performing the following required urinalysis/urine chemistry measurements using manual, automated, or semi-automated systems optimized and validated for rodent species:

- Urine appearance
- Urine volume
- Urine specific gravity or osmolarity
- Microscopic assessment of urine sediment
- Urine protein concentration
- Urine glucose concentration
- Urine creatinine concentration
- Urine enzyme activities as specified for individual studies (e.g., N-acetyl-β-glucosaminidase, lactate dehydrogenase, alkaline phosphatase, aspartate aminotransferase, and gamma glutamyl transferase)

7.4. Laboratory Requirements

The clinical laboratory scientist is responsible for the oversight of analysis and storage of clinical pathology samples and shall be available for consultation or questions. The training or approval of personnel to assume these tasks is also the responsibility of the clinical laboratory scientist, who shall review and sign off on data at the end of each day (or designate qualified personnel to perform this task).

The laboratory shall have in place all equipment necessary to perform (at a minimum) the aforementioned tests.

The laboratory shall have routine capability to collect and analyze blood, serum, and urine samples from rats and mice at a capacity of at least 60 samples of each sample type for each study day.

SOPs for performing the aforementioned clinical tests shall be made available for review and approval and submitted with the study file to the NTP Archives. The SOPs shall be accompanied by documented performance of the laboratory's ability to interpret results. Each laboratory shall maintain clinical pathology historical control data for each sex and species used, and those data shall be made available for review upon request.

Each laboratory performing clinical laboratory tests for NIEHS shall have written quality control procedures (i.e., SOPs) that are routinely followed and shall subscribe to a proficiency-testing program. In-house quality control procedures include scheduled equipment maintenance and calibration and cumulative records of performance utilizing normal and abnormal control reference materials or samples. Cumulative records of proficiency program testing results shall be maintained. Prior to approval to conduct clinical laboratory tests, 6-month cumulative data shall be submitted for evaluation; the data shall be in graph or tabular form.

The laboratory shall follow the criteria for rejecting sample runs based on the Westgard Rules (Westgard et al. 1981). The laboratory shall have written procedures for the application of the Westgard Rules and shall describe the criteria used for the determination of sample run acceptability. For quality control data, the source of control materials, the mean, and a measure of variability used in testing control materials to determine acceptability of sample runs must be maintained. If the mean and the measure of variability of commercially obtained controls were established in the testing laboratory, both in-house and manufacturer's values shall be maintained. The laboratory shall have written procedures for documenting incidents and deviations from the study protocol and SOPs. The degree to which deviations and incidents may influence the clinical laboratory measurements shall be identified. Examples of such deviations and incidents include: (1) technician error, (2) variation in reagent lots, (3) equipment drift or failure, and (4) the rejection of data of sample runs based on the concurrent analysis of control samples. Regardless of incident type, documentation of rejection and repeat of individual samples or sample runs must be made along with corrective action.

Protocol-required blood samples for hematology, clinical chemistry, etc. shall be obtained and analyzed in random order (not by dose group) for a given sex and species. The laboratory shall have written procedures for collecting and processing specimens in a randomized order. This requires an appropriate scheme for identifying and tracking specimens throughout all procedures.

Blood collection procedures shall be clearly identified. Blood collection sites, procedures, and anesthetics used shall be defined for both interim and terminal sampling. Unless otherwise specified by the study protocol, all terminal blood samples from rats and mice shall be collected from the retro-orbital sinus/plexus using a carbon dioxide/oxygen mixture as the anesthetic. In rats, survival (interim) blood collection shall be collected from the jugular vein without the use of anesthetic unless otherwise specified by the study protocol. All blood samples from the same study shall be collected from the same site. Sample volumes taken at interim bleeds shall not exceed 2.0% body weight for rats and 2.5% body weight for mice. Animals shall not be fasted prior to sample collection. For dermal, gavage, and inhalation studies, at each collection time point, animals shall be treated for a minimum of 2 consecutive days (within 24 hours) prior to sample collection. Animals are not to be treated on the morning of collection for these routes unless the protocol requires it. Blood samples for analysis in the clinical pathology laboratory shall be collected from the appropriate animals the morning of sample collection during a 3-hour period, including samples for analysis of routine hematology, clinical chemistry, urinalysis, and hormone variables. Methods for harvesting serum and plasma shall be described in the written procedures. All males and females of a given species shall be treated the same number of days before collection of samples, and all animals of a sex shall be bled on the same day. Perinatal and DART studies may require that blood collection occurs on a specific perinatal day (i.e., pups need to be exactly the same age) or gestation day (i.e., collection of blood from dams). Thus, blood collection may need to occur over several days instead of 1 day; in these studies, an appropriate scheme for the blood collection and analysis must be reviewed and approved by the program contracting officer's representative (COR). Overnight urine collection procedures must be clearly explained. As part of the demonstration of capability to collect blood and harvest serum, each laboratory must submit a listing of the total volumes per animal of whole blood, serum, and plasma that can be routinely obtained from rats and mice of 6 weeks, 17 weeks, and 6 months of age, using the retro-orbital bleeding technique. Both interim and terminal sacrifice volumes shall be listed.

If unsuitable blood samples are obtained from individual animals, those animals shall not be re-bled on subsequent days to fill the data gap. If the data gaps are significant, the program COR will determine if and when it may be necessary to re-bleed all animals.

Automated hematology measurements and blood smear preparations shall be made within 6 and 2 hours of sample collection, respectively. Constituents in serum (or plasma, if specified) shall be assayed the same day of sample collection. Samples collected for routine hematology assays (EDTA) shall not be stored on ice before analysis. During the period when the samples are not being assayed, they shall be kept tightly sealed at 4°C. With study-specific approval, samples (e.g., serum) may be frozen at −20°C or colder for subsequent analysis. The freezing of samples for storage prior to the performance of routine assays is not acceptable unless otherwise specified by the study protocol.

The laboratory shall have sufficient facilities for frozen storage of biological samples at −60°C or below. Such samples may be retained for up to 6 months following receipt of the relevant study report(s). Stained and cover-slipped peripheral blood, reticulocyte, and bone marrow smears shall be appropriately identified (as is the case with histology slide identification) and packaged for delivery to the NTP Archives at the conclusion of each phase of the study, as specified in Section 8.13.

7.5. Reporting Requirements

7.5.1. Submission of Unaudited Data

Summary tables—as well as individual animal data for all sample collections of routine chemistry assays, automated hematology analyses, and urine chemistry determinations—shall be submitted electronically to the program COR and pathology coordinator within 21 calendar days of sample collection. White blood cell differentials, reticulocyte counts, and morphologic evaluations of blood smears are to be included in the final report.

Data to be retained in the study file include original and repeat sample assays for individual animals. It is not necessary for these data to be subjected to internal quality assurance prior to submission.

7.5.2. Final Study Report

Summary clinical pathology data considered to be related to treatment shall be included in the "Results" section of the final report. All summary and individual animal data shall be organized by species, sex, and treatment group and included in the report appendices. Notations of any observation and/or action taken to confirm or explain atypical data points are to be included (e.g., dilution and reanalysis to confirm or establish values that exceed linearity of assay or reanalysis to confirm low values). Relevant comments concerning sample quantity (QNS) and quality (e.g., lipemia, hemolysis, icterus, etc.) are to be included. The methodology used to obtain samples and measure analytes must be described in the "Materials and Methods" section of the study report. Interpretation of the biological significance of the results shall be presented in the "Discussion" section of the study report and shall include correlations between clinical laboratory findings and anatomic pathological changes and/or clinical signs exhibited by the study animals.

7.6. References

Westgard JO, Barry PL, Hunt MR, Groth T. 1981. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem. 27(3):493-501. https://doi.org/10.1093/clinchem/27.3.493

7.7. Peer Review

The Division of Translational Toxicology (DTT) conducted a peer review of chapters 7 and 8 within the draft *Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences* by letter in February 2022 by the expert listed below. Reviewer selection and document review followed established DTT practices. The reviewer was charged to:

1. Peer review the following chapters within the draft Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences.
    - Chapter 7: Clinical Pathology
    - Chapter 8: Anatomic Pathology
2. Comment on the completeness of each chapter.

DTT carefully considered reviewer comments in finalizing this document.

Peer Reviewer

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