OECD and ARRIVE 2.0 Guidelines for Disease Model Testing in Rats

Introduction

This document outlines standardized workflow guidelines for disease model testing in rats, incorporating principles from the Organisation for Economic Co-operation and Development (OECD) Test Guidelines and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 Guidelines. These guidelines are designed to ensure scientific rigor, animal welfare, reproducibility, and transparency in disease model research using rats. The purpose of these guidelines is to provide researchers with a comprehensive framework for designing, conducting, analyzing, and reporting disease model studies in rats; to promote adherence to international standards for animal testing; to enhance the quality, reproducibility, and translatability of research findings; and to ensure humane treatment of laboratory animals in accordance with the 3Rs principle (Replacement, Reduction, and Refinement). The regulatory framework guiding these procedures includes OECD Guidelines for the Testing of Chemicals, ARRIVE 2.0 Guidelines, local and national legislation on animal experimentation, and Institutional Animal Care and Use Committee (IACUC) requirements.

Experimental Design

Study planning begins with clearly defining research questions and objectives, formulating specific, measurable, achievable, relevant, and time-bound (SMART) objectives that align with the 3Rs principles. A comprehensive literature review should be conducted to document previous findings related to the disease model, identify methodological considerations and limitations, and consider alternative methods that may reduce or replace animal use. Statistical planning involves performing sample size calculation based on expected effect size, desired power (minimum 0.8), and significance level (typically $\alpha = 0.05$), documenting the statistical methods to be used prior to experimentation, and considering sequential or adaptive designs to minimize animal numbers. Experimental groups should be defined including control groups (positive, negative, vehicle), with randomization procedures planned, blinding procedures implemented for treatment allocation and outcome assessment, and consideration of including both sexes unless scientifically justified otherwise. A detailed study timeline should be developed including acclimation, baseline measurements, disease induction, intervention, and endpoint assessment, with the duration defined based on disease progression and expected outcomes.

Pilot studies serve multiple purposes: validating disease induction techniques, refining assessment methods, determining appropriate endpoints, and estimating effect sizes for sample size calculations. Implementation of pilot studies should use minimal animal numbers needed for valid assessment, with all findings documented systematically and used to refine full study protocols. Protocol development involves creating detailed standard operating procedures (SOPs) for animal handling and care, disease induction methods, administration of test substances, sample collection techniques, assessment methods, and euthanasia procedures. Protocol review and approval requires submission of protocols for ethical review by IACUC or equivalent body, addressing all comments and concerns prior to study initiation, and ensuring all personnel are appropriately trained in protocols.

Animal Procurement and Husbandry

Animal selection requires documented scientific justification for using rats and the specific strain, selecting the strain based on disease model suitability and previous literature, and considering genetic background and potential confounders. Source and quality considerations include obtaining animals from reputable, certified suppliers, requesting health certificates and genetic quality assurance, and documenting vendor, breeding methods, and animal history. Age, sex, and weight considerations involve selecting age appropriate for the disease model (with documented justification), including both sexes unless scientifically justified otherwise (per ARRIVE 2.0), and documenting weight ranges while ensuring uniformity within experimental groups.

Housing specifications should detail cage type and dimensions (in accordance with national guidelines), number of animals per cage (with justification), bedding material and enrichment items, and cage changing frequency. Environmental parameters to be monitored and recorded daily include temperature (20-26°C, with <2°C variation), relative humidity (30-70%), ventilation (10-15 air changes per hour), light cycle (12:12 hour light:dark unless otherwise justified), and noise levels (<85 dB). Acclimation requires a minimum 5-7 day period before experimentation, habituation to handling and experimental procedures, and baseline physiological parameter measurement.

Nutrition considerations include using standard laboratory rodent diet (with documented manufacturer and product number), special diets if required by the disease model (with justification), feeding schedule (ad libitum unless otherwise justified), and quality control measures for feed. Water provision should address source and quality control measures, delivery method (bottles, automatic systems), and analysis of water quality if relevant to the study.

Disease Model Induction

Pre-induction preparations include documenting normal physiological parameters, conducting baseline behavioral assessments if applicable, and collecting baseline biological samples if needed. Equipment and material preparation involves verifying availability and functionality of all required equipment, preparing and validating test substances (documenting lot numbers, preparation methods), and preparing surgical areas if applicable. Personnel preparation ensures all staff are trained in appropriate techniques, with specific roles and responsibilities assigned, and personnel qualifications documented.

Chemical induction methods should detail substance preparation (concentration, vehicle, storage), administration route (oral, intraperitoneal, subcutaneous, etc.), dosing schedule and volumes, and safety precautions for personnel. Surgical induction requires anesthesia protocol (agent, dose, monitoring), aseptic technique procedures, surgical method details (with reference to established protocols), and post-operative care and analgesia. Genetic models need genotyping protocols and verification, breeding schemes if applicable, and special care requirements for specific genetic models. Behavioral induction involves standardized protocols for stress or other behavioral inducements, environmental modifications, and duration and frequency of exposure.

Post-induction care includes immediate monitoring with observation frequency and duration specified, physiological parameters to be monitored, and intervention criteria for unexpected responses. Recovery period management covers pain management protocols, supportive care measures, and criteria for intervention or removal from the study.

Monitoring and Assessment

General health monitoring involves daily observations including body weight measurement (frequency specified), food and water consumption, physical appearance and activity level, and fecal and urinary output. Score sheet implementation requires developing model-specific welfare assessment score sheets, defining scoring criteria for each parameter, establishing score thresholds for interventions, and training all personnel in consistent scoring. Documentation requirements specify standard format for recording observations, frequency of documentation, and review and sign-off procedures.

Disease-specific assessments include physiological parameters (blood pressure, heart rate, temperature measurement protocols, respiratory parameters if applicable, and model-specific physiological indicators), behavioral assessments (standardized tests appropriate for the disease model, testing schedule and environment, scoring and analysis methods, and habituation procedures to minimize stress), and functional assessments (movement/motor function tests if applicable, sensory function assessments if applicable, and organ function tests specific to the disease model).

Biological sample collection procedures should be detailed for blood sampling (methods such as tail vein, saphenous vein, cardiac, etc.; volumes and frequency with justification; processing and storage protocols; and maximum cumulative volume guidelines), urine and feces collection (collection methods such as metabolic cages or spot collection, duration and frequency, and storage and analysis protocols), and other biological samples (sampling techniques for specific tissues during the study, anesthesia requirements if applicable, and processing and storage procedures).

Data Collection and Management

Data recording systems may include electronic data capture (validated software systems, data entry procedures and verification, backup procedures and frequency, and access control and audit trail), paper records (standardized forms design, completion and review procedures, and storage and archiving), and data integration (methods for combining data from different sources, quality control checks, and consistency verification). Data quality assurance involves standard procedures (double data entry or verification methods, range checks and logical validation, and missing data identification and handling), calibration and validation (equipment calibration schedule and documentation, validation of measurement techniques, and inter-observer reliability assessment for subjective measures), and audit procedures (internal audit schedule and methods, external audit requirements if applicable, and documentation of audit findings and corrective actions).

Data security and privacy concerns should be addressed through access controls (authorization levels for different personnel, password protection procedures, and

secure storage of sensitive information) and data transfer protocols (secure methods for transferring data between systems, verification procedures after transfer, and documentation requirements).

Endpoint Determination

Humane endpoint criteria should be clearly defined, including model-specific humane endpoint definitions, general health-based criteria (e.g., weight loss >20%, severe distress), disease-specific progression criteria, and scoring system thresholds for intervention. The decision-making process should specify personnel authorized to make endpoint decisions, consultation procedures for borderline cases, and documentation requirements for endpoint decisions. Intervention procedures should detail immediate actions upon reaching endpoints, emergency treatment options if applicable, and euthanasia protocols.

Scheduled study termination considerations include timing (scientific justification for study duration, criteria for successful study completion, and procedures for staggered termination if applicable) and final assessments (terminal procedures specifications, final sample collection protocols, and documentation requirements).

Tissue Collection and Processing

Euthanasia methods should be approved techniques in accordance with AVMA guidelines, with confirmation of death procedures and documentation requirements clearly specified. Considerations include the impact of euthanasia method on tissue quality and study parameters, training requirements for personnel, and equipment maintenance and validation. Necropsy preparation details timing post-euthanasia, required equipment and materials, and personnel roles and responsibilities. Procedures include standardized necropsy protocols, gross examination and documentation, photographic documentation if applicable, and organ weight measurement protocols. Tissue collection covers prioritization of tissues based on study objectives, collection methods to avoid contamination or degradation, trimming and sectioning procedures, and labeling systems.

Tissue preservation and processing includes fixation (fixative selection based on planned analyses, fixation duration and conditions, and special processing for specific analytical methods), storage (fresh tissue handling such as flash freezing, fixed tissue storage conditions, labeling and inventory systems, and temperature monitoring for frozen samples), and processing for analysis (embedding procedures, sectioning protocols, staining methods, and quality control procedures).

Data Analysis and Reporting

Statistical analysis begins with pre-analysis procedures including data cleaning and validation, outlier identification and handling, testing statistical assumptions, and handling missing data. Analysis methods should detail justified selection of statistical tests, software and version documentation, analysis code preservation, and multiple comparison correction methods. Reporting requirements include complete reporting of all statistical methods, clear presentation of results with appropriate measures of

variability, reporting of effect sizes and confidence intervals, and transparent reporting of all analyses performed.

Results interpretation involves contextualization (interpretation in light of study hypotheses, comparison with previous findings, and discussion of limitations and potential confounders) and translational considerations (discussion of relevance to human disease, limitations of the model, and suggestions for further research). Reporting standards require ARRIVE 2.0 Guidelines compliance (completing all sections of the ARRIVE checklist, providing justification for any deviations, and including the completed checklist with publications), manuscript preparation (thorough methods description allowing replication, complete reporting of all animals and outcomes, transparent reporting of negative and positive results, and clear acknowledgment of funding sources and conflicts of interest), and data sharing (plans for sharing raw data, repository selection for data deposition, and metadata standards compliance).

Ethical Considerations and Regulatory Compliance

The ethical review process includes protocol submission (complete IACUC or ethical committee application procedures, addressing all required elements in the submission, and responding to committee questions and concerns), amendments and continuing review (procedures for protocol modifications, annual review requirements, and adverse event reporting), and documentation (maintaining approval documentation, recording all interactions with ethical committees, and documenting training and qualifications of personnel).

3Rs implementation covers replacement (documentation of consideration of non-animal alternatives, justification for animal use, and integration of in vitro or in silico methods where possible), reduction (sample size justification, use of appropriate statistical designs to minimize animal numbers, and tissue sharing and biobanking practices), and refinement (implementation of least invasive techniques, appropriate anesthesia and analgesia protocols, and environmental enrichment and welfare enhancement).

Compliance monitoring involves internal oversight (regular protocol compliance checks, documentation review procedures, and corrective action processes) and external inspections (preparation procedures for regulatory inspections, response procedures for findings, and documentation of all interactions with regulatory authorities).

Documentation and Record Keeping

Required documentation includes regulatory documents (ethical approvals and amendments, personnel qualifications and training records, standard operating procedures, and health and safety assessments), study-specific documents (study protocol and amendments, case report forms/data collection forms, raw data records, and analysis plans and results), and animal-specific records (individual animal identification and tracking, health monitoring records, intervention and treatment records, and endpoint and disposition documentation).

Records management systems should include secure physical storage for paper records, electronic records management system specifications, and backup procedures and frequency. Retention policies should specify minimum retention periods (according to regulatory requirements), archiving procedures, and retrieval mechanisms. Quality control involves regular auditing of documentation, correction procedures for errors, and validation of electronic systems.

Conclusion

Implementation of these comprehensive guidelines will ensure that disease model testing in rats is conducted with the highest standards of scientific rigor, animal welfare considerations, and regulatory compliance. By following these detailed procedures, researchers can enhance the reproducibility and translatability of their findings while minimizing animal use and distress. Regular review and updates to these guidelines are recommended to incorporate advances in the field and evolving best practices. The standardization provided by these guidelines contributes to global harmonization of animal testing procedures and supports the ultimate goal of developing effective treatments for human diseases.