CCPSE Guidelines for Testing Medical Device in Zebrafish Models

1. Pre-experimental Planning

The pre-experimental planning phase must begin with a comprehensive ethical review process following the principles outlined in OECD, ARRIVE 2.0, and CCPSE (Canadian Council on Animal Care Committee for Care and Use of Fish in Research) guidelines. All testing protocols must be submitted to the institutional animal care and use committee (IACUC) or equivalent ethical review body, with clear justification for using zebrafish and demonstration that alternatives or lower phylogenetic species are not feasible. Ethical approval must be documented with a reference number for future reporting, and all procedures must align with the 3Rs principle of Replacement, Reduction, and Refinement. The study design must define primary and secondary outcomes clearly while conducting appropriate sample size calculations based on power analysis that specifies effect size, power (typically 80% or 90%), and significance level (typically 0.05). Researchers should consider variability from previous studies when available and thoroughly document the methodology and tools used for calculation. Proper randomization procedures must be implemented using appropriate methods (simple, block, or stratified) and documented in detail, preferably utilizing computer-generated randomization when possible. Appropriate control groups must be included, such as negative control (sham operation), positive control (if applicable), and vehicle control (if applicable). Blinding procedures should be implemented wherever possible, with experimenters blinded to group allocation during procedures and outcome assessors blinded to treatment groups, with clear documentation when blinding is not possible and justification provided. Test item preparation requires detailed documentation of medical device specifications including physical dimensions, materials/composition, manufacturing details, and sterilization method, along with preparation of positive control items according to validated procedures and documentation of the chain of custody for all test items.

2. Zebrafish Selection and Housing

Zebrafish procurement must source animals from reputable vendors or in-house breeding facilities with established health monitoring programs, documenting source details, genetic background, and health status reports. Strain selection should choose the zebrafish strain most appropriate for the specific device testing with scientific justification documented and consideration of genetic background relevant to study endpoints, with transgenic or mutant lines fully characterized genetically. Complete genetic information including specific strain (e.g., AB, Tübingen, Casper) and generation must be reported. Sex considerations require inclusion of both male and female fish unless scientifically justified otherwise, with documentation of rationale if single sex is used and consideration of potential sex differences in the analysis plan. All sex-based differences in results must be reported. Age and size considerations require use of fish of appropriate developmental stage for specific endpoints (e.g., embryo, larva, juvenile, adult), documenting age in standardized units (hours post-fertilization [hpf], days post-fertilization [dpf], or months for adults), reporting standard length at study initiation (both range and mean ± SD), and considering developmental stage-related biological variables in study design. Housing conditions must maintain water temperature at 26-28.5°C (with daily fluctuations documented), pH at 7.0-7.5 (with daily monitoring), conductivity at 300-1500 μS/cm, and

implement appropriate light/dark cycle (typically 14/10 hours). Water quality parameters including ammonia (<0.02 mg/L), nitrite (<0.1 mg/L), and dissolved oxygen (>80% saturation) must be monitored regularly and documented, with maintenance of appropriate stocking density (typically 5 adult fish per liter for routine housing, and potentially lower during experimental procedures). Housing systems should be described in detail, including static, semi-static, or flow-through systems, water filtration methods, and tank dimensions, with feeding regimens (type, frequency, amount) documented and all housing parameters recorded in the final report.

3. Pre-experimental Procedures

Pre-experimental procedures must include an acclimation period of minimum 7-14 days before procedures for adult fish (shorter periods may be appropriate for embryos/larvae), with health parameters monitored during this time and handling and habituation protocols documented. Baseline measurements including length, weight (for adults), and clinical observations must be recorded. Pre-experimental fasting protocols should be implemented if required for adult fish (typically 12-24 hours), documenting fasting duration precisely, and recording pre-experimental measurements. A complete pre-experimental health assessment must be performed, documenting criteria for exclusion based on health status, recording baseline physiological parameters, and ensuring animals meet all inclusion criteria before proceeding with experimental interventions. For embryos and larvae, standardized staging must be performed according to established developmental criteria with careful synchronization of developmental timing between experimental groups to minimize variability in outcomes.

4. Anesthesia and Analgesia Protocols

Anesthesia and analgesia protocols for zebrafish require careful selection of anesthetics appropriate for the procedure and age of the fish, with MS-222 (tricaine methanesulfonate) being the most common, properly buffered to pH 7.0-7.5, at concentrations appropriate for the intended level of anesthesia (typically 100-200 mg/L for sedation, 150-200 mg/L for light anesthesia, and 200-300 mg/L for deep anesthesia in adult fish; concentrations should be adjusted for embryos/larvae). Alternative anesthetics such as eugenol (clove oil), etomidate, or 2-phenoxyethanol may be used with scientific justification, with doses, exposure times, and recovery protocols specified and documented. Anesthesia monitoring must assess depth of anesthesia using established parameters including loss of equilibrium, decreased opercular movement, and lack of response to touch stimuli, with monitoring frequency documented (continuous monitoring recommended), proper water temperature maintained during anesthesia, and detailed anesthesia records kept for each experimental group. While traditional analgesia protocols are less established for fish compared to mammals, potential pain or distress must be minimized through appropriate anesthetic depth during procedures, minimization of tissue damage, and implementation of optimal recovery conditions, with close monitoring for signs of distress post-procedure, including changes in swimming behavior, feeding, coloration, or social interactions, and modification of procedures if signs of distress are observed.

5. Experimental Procedures

Experimental procedures for device implantation or exposure must begin with proper preparation using aseptic technique appropriate for aquatic species, preparation of all equipment and tools, and documentation of all preparation procedures. The device exposure or implantation procedure must follow a standardized protocol documented in detailed step-by-step format, with precise anatomical location or exposure method recorded, any deviations from standard protocol documented, duration of procedure recorded for each experimental group, and detailed experimental records maintained. For microinjection procedures in embryos, standardized protocols for injection site, volume, and developmental stage must be specified. For implantation procedures in adult fish, precise surgical approaches must be documented with attention to anatomical landmarks. Monitoring during procedures requires continuous observation of fish during the procedure, documentation of any complications or adverse events, and maintenance of water quality and temperature within physiological range. Recovery procedures must place animals in clean, well-oxygenated recovery tanks with appropriate water quality, with continuous monitoring until normal swimming behavior is resumed, recovery timeline documented for each experimental group, post-procedure care protocol implemented immediately, and time to full recovery recorded.

6. Post-procedure Monitoring and Care

Post-procedure monitoring and care in the immediate post-procedure period (0-24 hours) requires monitoring fish at minimum intervals of 2-4 hours, assessing distress using validated observation methods that evaluate swimming behavior, opercula rate, coloration changes, and feeding behavior, documenting water quality parameters, implementing special feeding protocols if needed, and maintaining appropriate environmental conditions. Ongoing monitoring (>24 hours) must assess animals daily at minimum, document morphological changes, monitor the implantation site (if applicable) for inflammation, infection, or device migration, document feeding behavior, assess swimming and interactive behaviors, and record any signs of distress. Humane endpoints must be clearly defined with specific criteria for early euthanasia including persistent loss of equilibrium, inability to feed, signs of severe distress unrelieved by intervention, and specific complications related to the procedure, with the decision-making process for implementing endpoints documented, any unscheduled euthanasia recorded with detailed justification, and all early terminations reported in the final analysis.

7. Endpoint Assessments

Endpoint assessments must include regular observational assessments with systematic documentation of all behavioral observations, assessment of device function according to protocol, and recording of morphological parameters at defined intervals. For embryos and larvae, standard developmental endpoints such as hatching rate, cardiac function, spine curvature, swim bladder inflation, and touch response should be assessed as appropriate for the study. Physiological assessments should include measurements relevant to the device function, which may include heart rate, respiration rate, vascular flow, or specific organ function depending on the device being tested. Laboratory assessments may include collection of blood samples (for adult fish) or whole-body homogenates (for embryos/larvae) at predetermined timepoints for analysis of relevant biochemical parameters, with sample collection

procedures documented in detail and sample processing and analytical methods recorded. Molecular assessments may include gene expression analysis, protein quantification, or enzymatic activity measurements in specific tissues relevant to the device interaction. Pathology assessments require complete necropsy at study termination, with detailed external and internal examination, documentation of gross observations, collection of tissues according to standardized protocol, processing of tissues using validated histological methods, and detailed assessment of device-tissue interface, with local tissue response evaluated using standardized scoring systems for inflammation, fibrosis, necrosis, and foreign body reaction, potential systemic effects evaluated, and all procedures and findings documented in detail. Imaging assessments may include non-invasive imaging techniques such as fluorescence microscopy for transgenic reporter lines, optical coherence tomography, micro-CT, or other modalities appropriate for visualizing device-tissue interactions in zebrafish. Behavioral assessments should perform validated tests appropriate for endpoints, such as locomotor activity analysis, startle response, predator avoidance, shoaling behavior, or novel tank diving test, with testing protocols documented in detail, environmental conditions during testing recorded, and assessors blinded to treatment groups when possible.

8. Data Collection and Management

Data collection and management procedures require the use of standardized forms or electronic systems for all data collection that include experimental group ID, date, time, and observer for all records, documenting both raw data and derived values, maintaining source documentation for all measurements, and implementing quality control procedures for data entry. Image acquisition and analysis methods must be standardized and documented in detail, including equipment settings, analysis software, and quantification parameters. Sample management must label all samples with unique identifiers, document sample collection, processing, and storage conditions, maintain chain of custody for all samples, and record any deviations from sample handling procedures. Data management protocols should implement data validation procedures, document data entry verification processes, establish secure data storage systems, create regular data backups, and maintain an audit trail for any data modifications to ensure data integrity throughout the study.

9. Study Termination and Reporting

Study termination and reporting procedures must use euthanasia methods consistent with CCPSE and AVMA guidelines, typically using overdose of MS-222 (≥250 mg/L) followed by a secondary method such as pithing or rapid cooling for adult fish, with embryos/larvae requiring higher concentrations (typically ≥500 mg/L) or alternate methods such as rapid chilling for embryos <5 dpf. Euthanasia methods must be documented in detail (agent, concentration, exposure time), confirming death using appropriate secondary methods, and recording time and date of euthanasia for each experimental group. Statistical analysis must analyze data according to pre-specified statistical plans, document handling of missing data, address any protocol deviations in analysis, report both absolute and relative effects with precision estimates, present variability using standard deviation or confidence intervals, and include both raw data and derived values when appropriate. Reporting requirements following ARRIVE 2.0 guidelines must report study design details including sample size calculation,

randomization procedure, and blinding methodology; animal characteristics including strain, genetic background, sex, age, developmental stage, source, health status, and housing and husbandry conditions; experimental procedures detailing what was done, how it was done, when it was done, where it was done, and why it was done; and results showing numbers analyzed in each group, reasons for any exclusions, and outcomes and estimation with precision (e.g., confidence intervals), along with adverse events, protocol modifications, discussion of limitations and implications of findings, and statements on ethical approval and guidelines followed.

10. Quality Assurance

Quality assurance measures must include protocol compliance with regular monitoring of protocol adherence, documentation of any deviations with justification, implementation of corrective actions as needed, and maintenance of documentation for all monitoring activities. Personnel training requirements must document qualifications and training of all staff in zebrafish handling, anesthesia, experimental procedures, and euthanasia techniques, verify procedural competency before study initiation, implement standardized training for specialized procedures, and maintain training records for all personnel. Equipment calibration and maintenance procedures must calibrate all measurement equipment according to schedule, maintain service records for all equipment, document calibration procedures and results, and verify equipment function before critical measurements. Water quality monitoring equipment must be regularly calibrated and maintained according to manufacturer recommendations. Study documentation must maintain a complete study file including protocol and amendments, IACUC approval, standard operating procedures, raw data, analyses, correspondence, and final report, ensuring documentation meets GLP standards if applicable, and implementing secure archiving procedures for long-term data preservation and access.