1. PURPOSE

1.1 The purpose of this Standard Operating Procedure (SOP) is to describe the principles and criteria for the assay validation of bioanalytical methods for **small molecule drugs and therapeutic proteins** (e.g., monoclonal antibodies) used to support regulatory action based on bioanalytical data generated from **clinical pharmacology trials and non-clinical studies** including but not limited to: pharmacokinetic (PK), toxicokinetic (TK), bioavailability (BA), and bioequivalence (BE), conducted under Good Laboratory Practices (GLP) conditions and funded by the Division of Microbiology and Infectious Disease (DMID).

2. SCOPE

This SOP applies to the following clinical trials (2.1) and non-clinical studies (2.2) conducted under GLP and funded by DMID through a contract or cooperative agreement:

2.1 IND enabling clinical pharmacology trials (* with exception) including:

- First-in-Human (FIH)
- Single ascending dose (SAD) /Multiple ascending dose (MAD)
- Food- effect (FE)
- Bioavailability (BA)/Bioequivalence (BE) [*including IND exempt]
- Drug-drug interaction (DDI)
- Mass balance
- Thorough QT/QTc (TQT) or PK Concentration - QT assessment
- Special populations: pregnancy/nursing, pediatrics, older adults, renal impairment (RI), hepatic impairment (HI)
- Target site penetration into biological matrices: e.g. epithelial lining fluid (ELF), alveolar macrophages (AM), cerebrospinal fluid (CSF), cervicovaginal fluid, adipose tissue, urine.

2.2 Non-clinical studies are that intended to support a product approval under the Animal Efficacy Rule, and pivotal GLP toxicity studies.

3. DEFINITIONS

3.1 Audit (non-specific): A systematic, independent, and documented process for obtaining objective evidence and evaluating it objectively to determine the extent to which the audit criteria are fulfilled.

3.2 Clinical Trial: A research study in which one or more human subjects are prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of those interventions on health-related biomedical or behavioral outcomes.

3.3 Contract: A legal instrument by which a non-Federal entity purchases property or services needed to carry out the project or program under a federal award.

3.4 Regulatory Requirement: Obligatory requirement specified by an authority mandated by a legislative body.

3.5 Subject Matter Expert (SME): A person with bona fide expert knowledge about what it takes to do a particular job.

For other definitions, see DMID glossary.

4. RESPONSIBILITIES

4.1 Program staff responsibilities:

4.1.1 DMID staff (PO, COR, or SL) who have a role in ensuring bioanalytical assay validation activities of bioanalytical methods are responsible for ensuring these activities are completed in a compliant manner to support DMID funded clinical pharmacology and GLP nonclinical studies.

4.1.2 Program staff of any funding mechanism where a bioanalytical method validation is funded submits a request for review of method SOP and assay validation plans to ORA Subject Matter Expert (SME) and other SMEs as appropriate.

4.1.3 Program staff ensure that bioanalytical methods are validated in accordance with the Food and Drug Administration (FDA) 2018 guidance document, “Bioanalytical Method Validation” and the FDA 2022 guidance document, “M10 Bioanalytical Method Validation and Study Sample Analysis” (Listed in section 6 References).

4.1.4 Program staff ensure that bioanalytical testing laboratory is compliant with GLP requirements.

4.2 The ORA Director is responsible for assigning the appropriate ORA SME for review.

4.3 The ORA SME is responsible for reviewing the provided documents and providing comments to Program staff.

5. PROCEDURE

5.1 In general, the clinical phase (first participant, first dose) of a clinical pharmacology trial may not be initiated prior to successful completion of bioanalytical method validation.

5.2 Program will request the ORA Director to identify an ORA SME if not already assigned.

5.3 ORA Director assigns an ORA SME if not already assigned.

5.4 Program staff consults with ORA and other SMEs on objectives of clinical pharmacology trials and nonclinical pharmacology studies to determine when a bioanalytical method will require validation.

5.5 Prior to bioanalytical validation study initiation:

5.5.1 Program staff provides the following documents to the ORA SME for review and comment prior to validation study initiation.

- Bioanalytical method validation plan from the bioanalytical laboratory contractor
- Clinical and/or nonclinical study synopsis or protocol
- Any previous bioanalytical assay validation reports

5.5.2 ORA SME reviews the bioanalytical method validation plan and provides comments to Program staff. Program staff provides DMID comments to the contractor. Additional review cycle(s) may be repeated until consensus is reached.

5.5.3 Program staff in collaboration with ORA will perform a facility assessment, which may include an audit, to verify the suitability of the facility for performing the required work after a contract has been awarded, but before bioanalytical assay validation starts. DMID may perform additional audits and/or facility assessments after the start of the validation study, if deemed necessary.

5.6 Program staff, working with the bioanalytical laboratory, ensures that bioanalytical methods will be:

5.6.1 Fit-for-purpose, meaning the assays should be developed as appropriate for the intended use of the data and the associated regulatory requirements. Any bioanalytical data generated from non-clinical GLP studies under the Animal Efficacy Rule in support of regulatory action and pivotal toxicity studies must be validated.

5.6.2 Validated in accordance with the Food and Drug Administration (FDA) 2018 guidance document, "Bioanalytical Method Validation" and the FDA 2022 guidance document, "M10 Bioanalytical Method Validation and Study Sample Analysis" (Listed in section 6 References).

5.6.3 Performed according to GLP requirements.

5.6.4 If needed, immunogenicity testing (i.e., anti-drug antibody detection assays [ADA]) for **therapeutic protein products** will be validated in accordance with FDA 2019 guidance document, "Immunogenicity Testing of Therapeutic Protein Products- Developing and Validating Assays for Anti-Drug Antibody Detection" (Listed in section 6 References).

5.7 Post validation study execution:

5.7.1 ORA SME reviews the draft validation report and provides comments to Program staff. Program staff provides DMID comments to the contractor. Additional review cycle(s) may be repeated until consensus is reached.

5.8 Post Biological Sample Analysis:

5.8.1 Program will provide biological sample analysis report(s) (i.e., drug concentration data obtained from clinical trial or non-clinical study samples determined by using the validated bioanalytical methods) to ORA SME after the completion of sample analysis for review and comment. Program staff provides DMID comments to the contractor. Additional review cycle(s) may be repeated until consensus is reached.

5.9 Documentation:

5.9.1 ORA SME will retain final copies of 1) validation plan, 2) validation report, and 3) biological sample analysis report in "Bioanalytical Method Validation" library maintained by the ORA regulatory contractor.

6. REFERENCES

6.1 U.S. Code of Federal Regulations 21 CFR 58: Good Laboratory Practice for Nonclinical Laboratory Studies: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-58

6.2 International Conference on Harmonisation E6 (R2) "Good Clinical Practice": https://www.fda.gov/downloads/Drugs/Guidances/UCM464506.pdf

6.3 FDA Guidance for Industry: "Bioanalytical Method Validation" May 2018: http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf

6.4 FDA Guidance for Industry: "M10 Bioanalytical Method Validation and Study Sample Analysis" November 2022: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m10-bioanalytical-method-validation-and-study-sample-analysis

6.5 FDA Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products-Developing and Validating Assays for Anti-Drug Antibody Detection," Jan 2019: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-testing-therapeutic-protein-products-developing-and-validating-assays-anti-drug

7. APPENDICES

Not applicable

8. REVISION HISTORY

8.1 DMID-RA-SOP-00002 rev 01 is the original version of this SOP. The prior version of this procedure was A&N-Policy-001 Validation Requirements for Bioanalytical Methods.

9. ADDITIONAL INFORMATION

9.1 Document Lead: ORA Director

9.2 Posting externally: Yes