1. PURPOSE

1.1 The purpose of this Standard Operating Procedure (SOP) is to describe the requirements for the development, qualification, and validation of immune and non-immune assays for clinical studies.

2. SCOPE

2.1 This SOP must be used for all clinical trials where DMID is the IND sponsor and for non-IND clinical trials funded by a contract. For other types of clinical trials (IND and non-IND) and clinical studies, use of this SOP is strongly encouraged.

2.2 This SOP does not apply to the validation of bioanalytical methods for small molecule drugs and therapeutic proteins. Please refer to RA-SOP-00002 titled "Validation Requirements for Bioanalytical Methods in Support of Pharmacokinetic (PK) Studies."

2.3 This SOP applies to humoral and cellular immunological assays that are not commercially available and are not covered under Clinical Laboratory Improvement Amendments (CLIA) including but not limited to:

- Enzyme-Linked Immunosorbent Assay (ELISA)
- Toxin Neutralization Assay (TNA)
- Plaque Reduction Neutralization Test (PRNT)
- Microneutralization assay (MN)
- Hemagglutination inhibition assay (HAI)
- Neuraminidase inhibition assay (NAI)
- IgG subclass typing/identification
- Opsonophagocytic Activity (OPA)
- Enzyme-linked immunospot (Elispot)
- Flow Cytometry
- Bead-based multiplex immunoassay (e.g., Luminex)
- T cell proliferation
- Mixed Lymphocyte Reaction

2.4 This SOP and the stage specific assay development approach also applies to laboratory developed assays for quantitation of pathogens in clinical samples and includes non-immunological assays including, but not limited to:

- Quantitative Polymerase Chain Reaction (qRT-PCR)
- Tissue Culture Infectious Dose ($TCID_{50}$)
- Mycobacterial Growth Indicator Tube (MGIT) time to positivity (TTP)
- Thick and Thin Blood Smears for Malaria
- Inhibition of Liver Stage Development Assay (ILSDA)
- Growth Inhibition Assay (GIA)
- Standard Membrane Feeding Assays (SMFA)
- Transmission Reducing Assay (TRA) and Transmission Blocking Assay (TBA)
- DNA or RNA Sequencing

- Bacteriophage related assays

2.5 This SOP applies when a commercially available diagnostic assay is used outside of its approved indication or specimen-type, e.g., blood, urine, sputum, etc.

2.6 This SOP provides considerations for when External Quality Assessment (EQA) may be required.

3. DEFINITIONS

3.1 **Critical reagents:** Critical Reagents are requisite components of an assay that have direct impact on assay performance. Examples of critical reagents are reference standards, antibodies, recombinant proteins, cell lines, and quality controls.

3.2 **External Quality Assessment (EQA):** EQA is defined as a system for objectively checking the laboratory's performance using an external agency.

For other definitions, see DMID glossary.

4. RESPONSIBILITIES

4.1 **DMID Staff** (such as COR/PO, SL, CPM) responsibilities:

4.1.1 Ensure that clinical assay methods incorporate applicable stage-dependent principles and criteria in accordance with this SOP.

4.1.2 Coordinate the review of assay method development and associated documentation.

4.2 The **ORA Director** is responsible for assigning the appropriate ORA Subject Matter Expert (SME) to review relevant documents.

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

5. PROCEDURE

5.1 Program staff will request the ORA Director to identify an ORA SME, if one has not already been assigned.

5.2 ORA Director assigns an ORA SME, if one has not already been assigned.

5.3 For clinical trials/studies, Program Staff reviews the clinical protocol to identify the assays needed to support the study objectives.

5.3.1 All assays noted in primary and secondary objectives must be assessed for stage of assay development.

5.3.2 For exploratory objectives, use of this SOP is encouraged for any assays for which SOPs and other reports will be submitted to the FDA (or other regulatory agency).

5.4 For immune assays, Program Staff reference Appendices A (Immune Assay Development Levels for Product Development Stages and Endpoints) and B (Requirements for the 4 Levels of Immune Assay Development) and propose an assay development plan.

5.4.1 Appendix A provides guidance for pairing the stage of development of the assay with its intended use in supporting studies.

5.4.2 Appendix B lists criteria for the four Levels of Assay Development to ensure that the minimum requirements for each developmental stage are met for immune assays. Assay development is divided into four levels (in order of increasing stringency): 1- Developed 2- Optimized 3- Qualified and 4- Validated.

5.5 For non-immune assays, Program Staff reference Appendices C (Non-Immune Assay Development Requirements for Product Development Stages and Endpoints) and D (Requirements for the 3 Levels of Non-Immune Assay Development) and propose an assay development plan.

5.5.1 Appendix C provides guidance for pairing the stage of development of the non-immune assay with its intended use in supporting studies.

5.5.2 Appendix D lists criteria for the three levels of Assay Development to ensure that the minimum requirements for each developmental stage are met for non-immune assays. Assay development is divided into three levels (in order of increasing stringency): 1- Optimized 2- Qualified and 3- Validated.

5.6 Program Staff, in consultation with ORA SME and other SMEs, review study objectives and the proposed assay development plan. Based on this review, the required level of development for all proposed assays is agreed upon. The levels of assay development listed in Appendices A and C are the minimum levels of development required. Program may opt to develop an assay to a higher stage than required.

5.7 Prior to sample testing:

5.7.1 Program Staff confirm that the clinical assay laboratory is (1) compliant with Good Documentation Practices (GDP), and (2) meet Good Laboratory Practices (GLP) or Good Clinical Laboratory Practices (GCLP) requirements depending on the development stage of the assay.

5.7.2 Program Staff inform the testing laboratory of the level of development required for each assay. Program Staff confirm that the testing laboratory will use appropriate critical reagents for the assay and, will develop the assay to required level.

5.7.3 Consideration for requiring participation in EQA - When more than one laboratory will be performing the clinical testing, DMID may require those laboratories to participate in an EQA program for sample collection or specific test method(s), on an as-needed basis. EQA is a method that allows for comparison of a laboratory's testing to a source outside the laboratory. The ORA Director in consultation with Program Staff and ORA SMEs will determine the need for participation of a laboratory in an EQA program.

5.7.4 Program Staff request assay protocols and reports as listed in Appendices B and D, from the testing laboratory. Documents helpful for assay documentation shall be provided to laboratories upon request. Available documents are:

- DMID-RA-TMPL-00003 Assay SOP Content Template
- DMID-RA-GUID-00002 Technology Transfer Instructional Guidance
- DMID-RA-TMPL-00001 Assay Development Report Template

- DMID-RA-TMPL-00002 Assay Validation Protocol and Report Templates

5.7.5 SMEs review the assay SOPs, protocols and reports and provide comments to Program Staff.

5.7.6 Program Staff provide DMID comments to the testing laboratory. Additional review cycle(s) may be repeated until consensus is reached. The final assay documents must be signed off by person responsible for Quality oversight such as Laboratory Manager or Principal Investigator.

5.7.7 Upon receipt of finalized assay documents from the testing laboratory, Program Staff will notify the laboratory it may proceed with clinical sample testing.

5.7.8 The final assay documents [SOP, protocol(s), and report(s)] are retained by both Program Staff and the contract laboratory/facility in accordance with all applicable records retention policies (institutional, DMID and regulatory authority).

5.7.9 For IND clinical studies, Program Staff provide final assay documents to DMID Regulatory Affairs Manager (RAM) for regulatory submissions. (Note: FDA has been requesting assay documents).

6. REFERENCES

6.1 ICH Q2(R1) Guidance: Validation of Analytical Procedures: Text and Methodology Guidance for Industry, September 2021: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2r1-validation-analytical-procedures-text-and-methodology-guidance-industry

6.2 Bioanalytical Method Validation Guidance for Industry, MAY 2018

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry

7. APPENDICES

7.1 Appendix A: Immune Assay Development Requirements for Product Development Stages and Endpoints

7.2 Appendix B: Requirements for the 4 Levels of Immune Assay Development

7.3 Appendix C: Non-Immune Assay Development Requirements for Product Development Stages and Endpoints

7.4 Appendix D: Requirements for the 3 Levels of Non-Immune Assay Development

8. REVISION HISTORY

8.1 DMID-RA-SOP-00001 rev 01 is the original version of this SOP. The prior version of this procedure was A&N-Guidance-001 Assay Development/Qualification/Validation Requirements for Clinical Immunological Assays.

9. ADDITIONAL INFORMATION

9.1 Document Lead: ORA Director

9.2 Posting externally: Yes

Appendix A: Immune Assay Development for Product Development Stages and Endpoints

Intended Use *(type of trial OR type of study AND type of endpoint)*	**Minimum Assay Development Stage Required**
Screening Assay for prior exposure	Optimized (may require qualification or validation depending on the disease)
Phase 1 - Primary Endpoint	Optimized
Phase 1 - Secondary Endpoint	Optimized
Phase 1 - Exploratory Endpoint	Developed
Phase 2 - Primary Endpoint	Qualified
Phase 2 - Secondary Endpoint	Optimized
Phase 2 - Exploratory Endpoint	Developed
Phase 3 or 4 - Primary Endpoint	Validated
Phase 3 or 4 - Secondary Endpoint	Qualified
Phase 3 or 4 - Exploratory Endpoint	Developed

Appendix B: Requirements for the 4 Levels of Immune Assay Development

Assay Status	Developed	Optimized	Qualified[1]	Validated
Assay procedure				
Standard Operating Procedure (SOP)[2]	Y	Y	Y	Y
Development report	Y	Y		
Qualification plan or protocol			Y	
Qualification report			Y	
QA approved validation protocol				Y
QA approved validation report				Y
Positive and Negative Controls	Y[3]	Y	Y	Y
Reference Standard[4]		Y	Y	Y
If Qualitative Assay - Positive and Negative samples should be distinguishable	Y	Y	Y	Y
If Quantitative Assay, assess linearity		Y	Y	Y
Critical Reagents[5] are defined[6]	Y	Y	Y	Y
Reagent Qualification		Y	Y	Y
System Suitability criteria are defined		Y	Y	Y
Specificity				
Species specificity/Matrix effect		Y	Y[7]	Y[7]
Selectivity/Specificity			Y	Y
Accuracy				
Accuracy			Y	Y
Precision				
Precision-Repeatability			Y	Y
Precision-Intermediate Precision			Y	Y
Detection Limit				
Limit of Detection (LOD), if applicable				Y
Range and Quantitation Limit				
Range, Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ)			Y	Y
Robustness				
Robustness/Ruggedness		Y	Y[7]	Y[7]

[1]Qualified for fit-for-purpose.
[2]SOPs must list critical reagents, critical process steps, equipment and software and plate layouts.
[3]Reproducible (n ≥3) for Positive and Negative controls
[4]When available, recommend use of Reference Standard from designated national/international authorities.
[5]For example, critical reagents for ELISA could be coating antigen, secondary antibody, immune serum, etc.
[6]Defined means that at least one lot of the reagent has been acquired – prepared internally or purchased.
[7]These activities are required to be conducted at the listed stage if they have not been conducted previously.
Y=Yes and Blank=Does not apply

Attachment C: Non-immune Assay Development for Product Development Stages and Endpoints

Intended Use *(type of trial OR type of study AND type of endpoint)*	**Minimum Assay Development Stage Required**
Phase 1 - Primary Endpoint	Qualified
Phase 1 - Secondary Endpoint	Qualified
Phase 1 - Exploratory Endpoint	Optimized
Phase 2 - Primary Endpoint	Validated
Phase 2 - Secondary Endpoint	Qualified
Phase 2 - Exploratory Endpoint	Optimized
Phase 3 or 4 - Primary Endpoint	Validated
Phase 3 or 4 - Secondary Endpoint	Qualified
Phase 3 or 4 - Exploratory Endpoint	Qualified

Appendix D: Requirements for the 3 Levels of Non-immune Assay Development

Assay Status/ Minimum Requirements	**Optimized**	**Qualified[1]**	**Validated**
Assay procedure			
Standard Operating Procedure (SOP)[2]	Y	Y	Y
Development report	Y		
Qualification plan or protocol		Y	
Qualification report		Y	
QA approved validation protocol			Y
QA approved validation report			Y
Positive and Negative Controls	Y	Y	Y
Reference Standard[3]		Y	Y
Linearity	Y	Y	Y
Critical Reagents[4] are defined[5]	Y	Y	Y
Reagent Qualification	Y	Y	Y
System Suitability criteria are defined	Y	Y	Y
Specificity			
Species specificity/Matrix effect	Y	Y[6]	Y[6]
Selectivity/Specificity		Y	Y
Accuracy			
Accuracy		Y	Y
Precision			
Precision-Repeatability		Y	Y
Precision-Intermediate Precision		Y	Y
Detection Limit			
Limit of Detection (LOD), if applicable			Y
Range and Quantitation Limit			
Range, Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ)	Y	Y	Y
Robustness			
Robustness/Ruggedness	Y	Y[6]	Y[6]

[1] Qualified for fit-for-purpose.
[2] SOPs must list critical reagents, critical process steps, equipment and software and designs such as plate layouts.
[3] When available, recommend use of Reference Standard from designated national/international authorities.
[4] For example, critical reagents for ELISA could be coating antigen, secondary antibody, immune serum, etc.
[5] Defined means that at least one lot of the reagent has been acquired – prepared internally or purchased.
[6] These activities are required to be conducted at the listed stage if they have not been conducted previously.
Y=Yes and Blank=Does not apply