STATE OF NEW YORK
DEPARTMENT OF HEALTH

Wadsworth Center The Governor Nelson A. Rockefeller Empire State Plaza P.O. Box 509 Albany, New York 12201-0509

ASSAY APPROVAL IN CELLULAR IMMUNOLOGY

Please submit all information as outlined below. Submit one hard copy of the entire package and one electronic copy (as a PDF file on a CD or flash drive) to: **Clinical Laboratory Evaluation Program, Wadsworth Center, New York State Department of Health, P.O. Box 509, Empire State Plaza, Albany, NY 12201-0509; Attn: Assay Validation Review.** Materials submitted, including related data packages, cannot be returned to the laboratory. All materials are maintained under strict confidentiality. As relates to New York State's Freedom of Information Law (commonly called FOIL): The Department's Records Access Officer has advised Wadsworth Center that if documents are marked "proprietary"; "confidential"; or with any labeling indicative of the submitter's desire for an increased level of protection based on the submission content, such protection from immediate release based on a FOIL request is justified. Laboratories will be given an opportunity to block information release if a request for the material is filed under the FOIL, by presenting evidence that the materials contain trade secrets. Marking should minimally appear on the cover page of each unit of material. Documents not marked with such terms will not block release of the submission through a FOIL request.

SECTION 1: GENERAL INFORMATION

Laboratory Name: _____NYS PFI: _____

Contact Person: _____

Phone: _____ Fax: _____ Contact E-mail:_____

Assay (Test) Name (e.g. lymphoid function; PNH; malignant immunophenotyping):_____

Methodology (e.g. mitogen stimulation; CD55/59 flow-cytometric immunophenotyping):

_____

Analyte(s) included (if different from Assay Name): _____

Validated Specimen Type(s): _____

Clinical Purpose: _____

Laboratory Director/Assistant Director (NYS Certificate of Qualification Holder for Cellular Immunology Category)

CQ Code _____ Signature _____

Laboratory Director (if not the responsible CQ Holder for the Cellular Immunology Category)

CQ Code _____ Signature _____

Guidance for Cellular Immunology Submissions:

Submission and validation requirements vary dependent on the type and methodology of assay. The following guidelines define comprehensive requirements for validation and submission (where applicable) of new or modified Cellular Immunology assays. In addition, please review both the General Systems and the Cellular Immunology sections of the New York State Clinical Laboratory Standards of Practice, available at www.wadsworth.org/labcert/clep/standards.htm. Technical questions regarding the classification or requirements for validation of an assay that falls under the category of Cellular Immunology, but which is not listed here, can be addressed by calling (518) 408-2107.

There are three categories of testing covered under Cellular Immunology:

Category	Description
Leukocyte Function	All functional assays that involve *in vitro* testing of lymphoid, monocytic, and myeloid cells are included in this category. The following are examples of assays included in this category: • Proliferation assays (mitogen, antigen, or alloantigen stimulation) • Cytolytic assays • Cytokine or immunoglobulin production • Chemotaxis • Adherence/adhesion • Phagocytosis • Oxidative burst • Degranulation
Non-Malignant Leukocyte Immunopheno-typing	This category covers immunophenotyping for the identification and the enumeration of non-malignant leukocyte populations. The following are examples of phenotyping included in this category: • Lymphoid immunophenotyping • T-lymphoid immunophenotyping (restricted to CD3/CD4 and CD3/CD8) • Stem cell analysis • PNH analysis • LAD analysis
Malignant Leukocyte Immunopheno-typing	This category includes the identification and characterization of leukemias or lymphomas in blood and tissue specimens based on the cell phenotyping. This includes both cell surface and cytoplasmic antigens, with or without DNA ploidy determination.

SECTION 2: PLEASE REFER BELOW FOR SUBCATEGORY-SPECIFIC REQUIREMENTS.

All assays require submission of a comprehensive standard operating procedure and supporting validation materials, unless otherwise noted. When adding the category of Cellular Immunology to a new or existing permit, on-site survey is required. An on-site survey is not required for each assay prior to patient testing if the laboratory holds the applicable permit category.

Leukocyte Function

- Values listed are the minimum sample numbers required.
- Normal ranges annual upkeep: routine testing (RT)≥ 50 test/yr.; non-routine testing (nonRT)< 50 test/yr
- Validations for equipment changes and lab relocations assume no change to the assay; otherwise follow guidance for Modified Assay Validation, see Section 3.4.
- *Note 1*: Approval of proliferation assays will be final only after successful submission of the SOP and validation materials, and the successful participation in a New York State proficiency test for lymphoid proliferation.

	Submission for review and approval is required using these Guidelines and Checklist.				Maintain documentation for review during on-site inspection.		Submit *Notification of Change in Laboratory Location* form to CLEP. Maintain validation materials for review during on-site inspection
	Submit SOPM?	***New Assay Validation*** *(total / abnormals)*	***Modified Assay Validation*** *(total / abnormals)*	***Normal ranges***	***Normal ranges- Annual upkeep*** *RT / nonRT*	***Validation for Equipment Changes*** *(normals only)*	***Validation for Lab Relocation*** *(normals only)*
Proliferation[1] including Mitogen; Alloantigen; or Antigen (baseline and for each stimulant)	yes	25/5	25/5	25	25/ Each run	5	5
Cytokine production (per cytokine; baseline and for each stimulant)	yes	25/5	25/5	25	25/ Each run	5	5
Cytolytic Activity (per effector type and target)	yes	25/5	25/5	25	25/ Each run	5	5
Chemotaxis (per chemokine)	yes	25/5	25/5	25	25/ Each run	5	5
Adherence/ Adhesion (per biomarker; baseline and for each stimulant)	yes	25/5	25/5	25	25/ Each run	5	5
Phagocytosis (per effector and target)	yes	25/5	25/5	25	25/ Each run	5	5
Oxidative Burst (per ROS probe; baseline and for each stimulant)	yes	25/5	25/5	25	25/ Each run	5	5
Degranulation (per released product)	yes	25/5	25/5	25	25/ Each run	5	5
Other:	yes	25/5	25/5	25	25/ Each run	5	5

Non- Malignant Leukocyte Immunophenotyping

- Values listed are the minimum sample numbers required.
- Normal ranges annual upkeep: routine testing (RT)≥ 50 test/yr.; non-routine testing (nonRT)< 50 test/yr
- Validations for equipment changes and lab relocations assume no change to the assay; otherwise follow guidance for Validation for Assay Modification, see Section 3.4.
- *Note 1*: Approval of Lymphoid and T-lymphoid Immunophenotyping assays will be final only after successful submission of the SOP and validation materials, and the successful participation in the appropriate New York State proficiency test.
- *Note 2*: If the assay is FDA-cleared/approved and used without modifications, submission of the SOP and validation materials is not required; however validation/verification materials should be available for review during on-site inspection.

	Submission for review and approval is required using these Guidelines and Checklist.				Maintain validation for review during on-site inspection.		Submit *Notification of Change in Laboratory Location* form to CLEP. Maintain validation materials for review during on-site inspection
	Submit SOPM?	***New Assay Validation*** *(total / abnormals)*	***Modified Assay Validation*** *(total / abnormals)*	***Normal ranges***	***Normal ranges- Annual upkeep*** *RT / nonRT*	***Validation for Equipment Changes*** *(normals only)*	***Validation for Lab Relocation*** *(normals only)*
Lymphoid Immunophenotyping[1,2] (per subset)	yes	25/5	25/5	25	25/ Each run	5	5
T-Lymphoid Immunophenotyping[1,2] (per subset)	yes	25/5	25/5	25	25/ Each run	5	5
PNH analysis (per biomarker)	yes	10/5	10/5	25	25/ Each run	5	5
LAD analysis (per biomarker)	yes	25/5	25/5	25	25/ Each run	5	5
Other:	yes	25/5	25/5	25	25/ Each run	5	5

Stem Cell analysis[1,2]

- Values listed are the minimum sample numbers required per specimen type to be tested by the laboratory.
- *Note 1*: Approval of Stem Cell assays will be final only after successful submission of the validation materials and the successful participation in the New York State proficiency test for $CD34^+$ stem cell quantification.
- Note 2: Please complete and submit the Add/Delete FDA-Approved Test form for each stem cell assay. Full submission of the validation materials for FDA-cleared/approved tests used without modification is not required, but all documentation must be available for review during on-site inspection. All other stem cell tests require full submission of validation materials.

Submit SOPM?	***New Assay Validation*** (per specimen type)	***Validation for Assay Modification*** (per specimen type)	***Validation for Equipment Changes*** *(multilevel commercial controls)*	***Validation for Lab Relocation*** *(multilevel commercial controls)*
Yes	5	5	5	5

Malignant Leukocyte Immunophenotyping

- Values listed are the minimum sample numbers required.
- Validations for equipment changes and lab relocations assume no change to the assay; otherwise follow guidance for Validation for Assay Modification, see Section 3.4.
- *Please note:* Approval of malignant leukocyte immunophenotyping assays will be final only after successful submission of the SOP and validation materials, and the successful participation in the appropriate New York State proficiency test.

	Submission for review and approval is required using these Guidelines and Checklist.				Maintain validation for review during on-site inspection.		Submit *Notification of Change in Laboratory Location* form to CLEP. Maintain validation materials for review during on-site inspection
	Submit SOPM?	***New Assay Validation*** *(normals/ abnormals*	***Modified Assay Validation*** *(normals/abnormals)*	***Normal ranges***	***Normal ranges- Annual upkeep***	***Validation for Equipment Changes*** *(normals)*	***Validation for Lab Relocation*** *(normals)*
Malignancy Panels (per panel)	yes	5/5	5/5	25	10	5	5

SECTION 3: COMPLETE THIS ENTIRE SECTION AND PROVIDE ALL REQUIRED ATTACHMENTS FOR ASSAYS INDENTIFIED ABOVE REQUIRING SUBMISSION OF ALL VALIDATION MATERIALS

Please submit the following information, organized with an index or table of contents, as numbered or tabbed attachments as indicated below. If an item is not included, indicate the reason. Indicate the **page numbers and/or tabs where** the items and/or attachments can be found. **SUBMISSIONS THAT ARE NOT ORGANIZED AS DESCRIBED MAY BE RETURNED AND THE REVIEW SIGNIFICANTLY DELAYED.**

Section 3.1: Standard Operating Procedures Manual (SOPM)

The Standard Operating Procedure (SOP) should be written so that any technologist (after training) can refer to the manual to perform the assay and properly describe the results to produce the report for accurate interpretation of the analysis. An acceptable format should be similar to that found in the Clinical and Laboratory Standards Institute (CLSI) guidelines GP02-A5. The final document must include all requirements detailed in the NYS Clinical Laboratory Standards of Practice, SOPM S2 standard.

Clearly written procedures for laboratory-developed or modified commercialized assays (FDA-cleared/approved or non-FDA cleared/approved) are essential for proper test performance. Submission and validation requirements vary dependent on the type and methodology of assay.

Page/Tab	**General SOPM Requirements**
	The intended purpose and principle of the assay
	Specimen requirements, including the following: • Requirements for patient preparation prior to sample collection • Appropriate anticoagulant(s) for blood and bone marrow specimen collection and saline/media for tissue stability post collection • Acceptable sample age, temperature, and quantity • Storage and handling requirements • Rejection criteria and procedure
	A complete reagent listing including the source and catalog number for all reagents and complete preparation, storage, shelf life, and handling instructions.
	A complete listing of the required equipment and supplies. (Centrifuge and rotor heads must be identified, or the required g-force must be delineated in the instructions.)

	Quality control procedures for the assay, including assay and equipment calibration and QC requirements for assay acceptability
	Step by step procedure for performing the assay including the use of methodological controls
	Instructions for reporting results including calculation of the results, reportable ranges, and procedures in the event of abnormal or flagged results
	Any miscellaneous information pertinent to the performance of the assay, including limitations, influencing factors, sources of errors, and special precautions
	Method references
	Leukocyte Function Assay SOPM Requirements *Please review Cellular Immunology Standards CI6 – CI18 to ensure procedural compliance with all standards.*
	Inclusion of a positive control from a healthy donor in each assay run and/or each assay plate
	Instructions for checking the viability of the specimen(s). Noting the degree of viability necessary for acceptability and the basis for the cursor setting defining viable from non-viable.
	Assay-specific quality control requirements
	For assays that are analyzed via flow cytometry, complete instructions for instrument setup and sample acquisition and analysis must be provided for review
	Non-Malignant Leukocyte Immunophenotyping Assay SOPM Requirements *Please review Cellular Immunology Standards CI22- CI43 to ensure procedural compliance with all standards.*
	Quality control procedures for setup and maintenance of the flow cytometer
	Anticoagulant-specific specimen age guidelines (some assays may have shorter specimen age requirements) • 30 hours for specimen collected in tri-potassium EDTA • 48 hours for specimens collected in ACD or sodium heparin
	Guidelines for the acceptable time limits between staining, analysis, and storage of (fixed) samples
	Instructions for the acquisition and analysis of samples, including the minimum number of events that must be collected and appropriate population gating
	Assay-specific QC requirements
	Malignant Leukocyte Assay SOPM Requirements *Please review Cellular Immunology Standards CI44- CI58 to ensure procedural compliance with all standards.*
	Quality control procedures for set-up and maintenance of the flow cytometer
	Specimen acceptance guidelines including instructions for verifying the viability of the specimen after specimen processing into single cell suspensions
	Instructions for the acquisition and analysis of samples, including the minimum number of events that must be collected and appropriate population gating
	Analysis QC requirements

Section 3.2: Requisition and Reporting

Page/Tab	General Requirements
	A sample requisition
	Example reports should be provided in the submission demonstrating typical reporting conditions for the type of assay, minimally providing examples of an abnormal/positive result and normal/negative result. To be in compliance with New York State Clinical Laboratory Regulation Subpart 58-1.11 and the Post Examination Standard, Reporting Sustaining Standard of Practice 1 (Reporting S1): Report Content, all laboratory reports must include the following information: • Patient name and other identification (DOB, medical record number, etc.) • The name of the person or institution referring the specimen • Laboratory name and address • The date and hour when the specimen was originally collected by the physician or other authorized person • The date and hour the specimen was received in the testing laboratory • The date the specimen was tested • The result of the laboratory test or test(s) • Normal values, reference intervals, or similar method for identifying normal values
	Leukocyte Function Report Requirements
	Reports must include: • Patient identifiers • Specimen information • Date and time of sample receipt • Viability of specimens prior to the start of the assay • Interpretation of the sample response
	Non-Malignant Leukocyte Immunophenotyping Report Requirements
	Report must include: • Patient identifiers • Specimen information • Marker results • Result interpretation (or out of range indicators) • Normal range (% and/or absolutes values)
	Malignant Leukocyte Immunophenotyping Report Requirements
	Report must include: • Patient identifiers • Specimen information • Viability of specimen prior to flow cytometric analysis • Marker results • Diagnosis or characterization including lineage and stage

Section 3.3: References and Support Documents for Testing

Page/Tab	General Requirements
	Copies of references listed in the standard operating procedure
	Copies of the current package inserts for the assay's commercially distributed test kit(s) and/or reagent(s)
	Practitioner and patient educational materials
	Clinical indications for testing, including, where appropriate, the prevalence and description of the medical condition
	Copy of client instructions for specimen collection and transportation requirements

Section 3.4: Validation Protocol and Data

The validation data should include the analysis of normal and abnormal specimens that are collected to establish assay performance and verify the normal reference range for a particular parameter, e.g., absolute number of $CD4^+$ T cells. The minimum number of specimens required for validation of an assay vary dependent on the assay; for detailed information on the number of samples required please refer to the **Category Requirements Table** above.

Requirements for newly developed assays-

- Laboratory-derived normal ranges from "healthy" individuals similar to the expected patient population in age, sex, and ethnicity.
- The determined reference (normal) range should be similar to published values, and the expected values obtained using samples from healthy individuals. (For example, "normal adult peripheral blood lymphocytes should have $CD4^+$ cells but few or no $CD10^+$ cells.)
- For assay validations, abnormal specimen submissions may include samples from qualified proficiency test providers, split samples from a CLIA-approved laboratory, and/or samples generated in-house. If the abnormal samples are generated in-house, the submission must clearly state how these specimens were generated and tested. However, it is preferred that the abnormal samples are obtained from either PT or split sample analysis. The submission must include data analysis (minimally instrument print-outs) in addition to example patient reports for these analyses.

Requirements for modifications to previously established/approved laboratory assays-

- Split (normal and abnormal) sample analysis demonstrating performance utilizing the old and the revised procedure.
- Results from the two assays should produce comparable patient outcomes. If not, the laboratory must calculate new normal ranges for the new procedure and indicate on patient reports that a new procedure has been implemented (include date), which could result in a non-biological change in the patient's longitudinal status.

Requirements for equipment/relocation validations-

- Normal specimens analyzed after the equipment/relocation changes must agree with previously determined laboratory-derived normal ranges. If not, the laboratory must calculate new normal ranges and indicate on patient reports that a procedural change has occurred (include date), which could result in a non-biological change in the patient's longitudinal status.

Pre-examination

Please note: Validations for specimen age cut-offs post collection require comparison with fresh specimens (0-4hr) to determine the laboratory's delay prior to testing analysis. A minimum of five normal specimens and five abnormal specimens shall be used to make these specimen integrity determinations and all data shall be submitted for section review.

Page/Tab	
	Analyte and specimen matrix stability
	Specimen transport conditions
	Storage time and temperature

Examination

Please note: assay validation requirements are listed below for both Leukocyte Function and Flow Cytometric Immunophenotyping assays. In the event there is overlap (e.g., a functional assay that is analyzed by flow cytometry) both sets of validation requirements apply.

Page/Tab	
	Leukocyte Function Assays
	A) **Accuracy** i) Verification must be supplied for a normal specimen showing that the result obtained falls within the established normal range, which is in agreement with published reference ranges. In addition, the initial established normal range must be comparable to that reported for similar methods and population demographics.
	B) **Precision** i) Intra-assay: five specimens using three replicate analyses. For microplate-based assays replicate analyses for each sample should be contained on the same microplate.
	ii) Inter-assay precision: five specimens assayed in singlicate over five separate assays. For microplate-based assays, these five specimens should be analyzed by plating the same samples on five different plates and evaluating the results for plate to plate differences in the results.
	C) **Reportable Range** i) Values for both the lower end (unstimulated values) and upper end (maximum values) of the reportable range must be determined. If applicable, the saturation value for a particular assay must also be determined.
	D) **Reference/Normal range verification** (Please refer to the **Category Requirements Tables** for additional information) i) Reference ranges should reflect the target population (e.g., child vs adult).
	ii) Annual reference range verification is required.
	E) **Analytical Sensitivity** (refer to Section 2 for additional information) i) The minimum number of cells required for each assay and/or stimulant must be determined to achieve a statistically significant difference between the unstimulated and stimulated results.
	F) **Analytical Specificity** (refer to Section 2 for additional information) i) In those assays in which measurement of a particular analyte is used as the result (e.g., Cytokine Production assays), validation of the assay must demonstrate that there is <5% cross-reactivity in the measurement of the target analyte.
	Flow Cytometric Immunophenotying Assays (Non-malignant and Malignant)
	A) **Accuracy** i) Commercial controls (multi -levels, if possible) when available of the same matrices; results must be consistent with manufacturer's assayed value ranges
	ii) Laboratory derived normal sample value(s) within the normal range of appropriate and consistent, published, normal ranges (pediatric *vs*. adult *vs.* geriatric)

	B) **Precision** i) Instrument Precision (Intra-assay) - one stained tube analyzed with ten replicate runs (to be completed for new assay or modifications to existing assays, additions &/or changes to equipment, after any major repair, and during parallel instrument testing)
	ii) Assay Procedural Precision (Intra-assay and Inter-assay) - five specimens using three replicate analyses for minimum and maximum time period for sample analysis (to be completed for new assays or modifications to existing assays.)
	iii) Staff Procedural Precision (Inter-assay and Intra-assay) - five specimens using three replicate analyses for minimum and maximum time period for sample analysis per employee responsible to complete assay testing (to be completed as part of post-training competencies, and annual competency assessments thereafter)
	C) **Reportable Range** i) Reliable flow cytometric analyses are dependent on adequate and appropriate population event collection to enable accurate reportable results. Recommendations for event count number and population gating method(s) from accepted published references and text, in addition to reagent product inserts, shall be used.
	D) **Reference/Normal range verification** (refer to Section 2 for additional information) i) new assays or modifications to existing approved assays: laboratory derived normal range development (pediatric *vs*. adult *vs.* geriatric)
	ii) annual range verification assessments
	E) **Analytical Sensitivity** (not required for FDA-Approved/cleared assays; refer to Section 2 for additional information) i) Analysis of specimens with high, intermediate and low biomarker expression of analyte for known disease state. Multiple levels abnormality should be ascertained and results should be representative of such condition.
	ii) Known normal specimens should demonstrate “normal” phenotype.
	F) **Analytical Specificity (**not required for FDA-Approved/cleared assays; refer to Section 2 for additional information) i) new assays - split sample analysis of abnormal specimens with a NYS permitted lab (with analyte approval), demonstrating comparable patient outcomes
	ii) modifications to existing NYS-approved assays - inter-laboratory split sample analysis (including normal and abnormal specimens), demonstrating comparable patient outcomes between the current assay and the modified assay.

Page/Tab	**Post-Examination**
	QC review process for the determination of reportable results
	Data reduction and result interpretation
	Reference interval format and usage toward all potential results
	Representative Specimen Runs
	All data used for specimen integrity determinations (pre-examination requirements including specimen age cut-offs)
	The data and the corresponding example result reports of minimally the five abnormal/positive specimens and five normal/negative specimens. (A larger data submission may be determined to be necessary after review of the smaller data set.)