Cellular Immunology

Standard	Guidance
All laboratories shall comply with the applicable requirements contained in the Clinical Laboratory Standards of Practice- General Systems. In addition, the Cellular Immunology laboratory shall meet the applicable standards outlined below:	Assay Validation Requirements: Validation requirements, including, specimen number, are available from the Cellular Immunology Submission Guidelines for Assay Approval (http://www.wadsworth.org/labcert/TestApproval/forms/Cellular_Immunology_Checklist.pdf) Laboratories can view the status of their validation packages on their EPTRS web page.

Leukocyte Function

Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 1 (CILF S1): Client Instructions for Specimen Collection and Transport The laboratory shall provide specimen collection and transport instructions to clients indicating: a) the anticoagulant(s) that must be used for each assay offered; b) the specimen's collection date and time must be documented; c) blood samples must be handled and transported in a manner to assist in maintenance of the specimens between 18 and 25 degrees Celsius and transported in a timely manner so that the laboratory can begin testing within the NYS-approved validated assay time frames; d) specimen collections must be performed at the same time of day when longitudinal studies of function involving serial monitoring have been requested; and e) any other information considered significant for specimen analysis.	It is recommended that a specimen from a normal donor be collected and shipped whenever possible, as a shipment control. Comparison of the shipment normal control and the in-house derived normal control should assist in controlling for possible shipment effects. e) For example, required specimen volume for testing (refer to Leukocyte Function Standard 5).

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 2 (CILF S2): Specimen Anticoagulant Requirements The laboratory shall only accept and analyze blood provided in the anticoagulant that has been used to establish and standardize the assay and achieve normal ranges, as previously approved by NYS.	If a specimen is received in an anticoagulant that is different than that defined in the specimen collection instructions, the specimen must be rejected unless the laboratory has data substantiating that a substitute reagent does not produce a statistically different response.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 3 (CILF S3): Specimen Storage Length and Temperature The laboratory shall: a) test blood specimens that are 24 hours or less post-collection unless NYS-approval for testing older specimens has been obtained; b) store specimens post collection between 18 and 25 degree Celsius with minimal exposure to temperature fluctuations.	a) Some functional assessments may require shorter specimen storage length dependent on the function to be assessed. Specimen storage before assay set-up should be kept to a minimum; 8 hours or less is optimal. Validations should demonstrate that results are within 10 percent of time zero (0-4hr) analysis. b) Specimen functional performances are very sensitive to temperature, and fluctuations can cause erroneous results.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 4 (CILF S4): Specimen Viability A viability assessment shall be performed from an aliquot of the whole blood specimen before the assay or cell culture is started. a) Specimens with cell viability less than 80 percent shall be rejected unless the specimen is deemed irreplaceable or a specimen re-draw would be harmful to the patient. b) The viability percentage shall be included in the laboratory report. c) The report shall include a disclaimer when reporting an irreplaceable specimen with a viability of less than 80 percent.	In the event that the specimen is irreplaceable or cannot be re-drawn, criteria must be included in the laboratory SOP to delineate how the patient specimen should be handled.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 5 (CILF S5): Specimen Adequacy The procedure shall define the amount of specimen (volume or cell number) needed to report test results.	Client instructions should convey individual assay requirements for specimen volume or cell number required to perform testing. Adequate numbers and viability of the cell type needed for the assay should be known prior to making the requested analysis.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 6 (CILF S6): Aseptic Reagents The laboratory shall establish and implement a procedure for ensuring use of contamination free medium and other reagents.	Laboratories that choose not to routinely use antibiotics in cultures should document that only aseptic reagents are utilized by using routine evaluation for signs of contamination. Laboratories that use commercially prepared media should retain the manufacturer's documentation that each shipment or lot of media has been subjected to appropriate quality control procedures. The user should visually examine each shipment for color and opacity as well as ability to support cell viability and growth. In-house produced media should include analysis of aseptic condition and adequate cell growth upon stimulation.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 7 (CILF S7): Limit of Pyrogene Levels All components (media, reagents, and plastic-ware) of the assay procedure involved with the measurement of functional activity shall not exceed 0.5 endotoxin units (EU)/mL).	The laboratory may accept the manufacturer's documention of acceptable levels of endotoxin. The laboratory must know the level that the manufacturer considers acceptable, but should not exceed 0.5 EU/mL.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 8 CILF S8): Serum Component of Media If human serum is used for the functional assay, AB serum shall be used.	Red cells and red cell membranes are common contaminants of extracted cellular material. Use of other blood type plasma could cause agglutination, if mismatched.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 9 (CILF S9): Reagent Verification Lot-to-lot evaluation of all reagents shall use a normal control specimen to ensure reagents give results within laboratory-derived reference range values.	Lot-to-lot checks, which include all steps of specimen processing, may be used for twice a year accuracy checks of the assay if there is no external proficiency testing program available. These checks may also be used as a competency assessment of staff.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 10 (CILF S10): Biological Safety Cabinet Requirement The laboratory shall use aseptic techniques during all steps of cell culture set-up and manipulation using a biological safety cabinet (BSC).	The use of a biological safety cabinet is imperative to reduce environmental contamination and to reduce risk of exposure to any potential pathogen. Refer to General Systems Safety Standards for operational requirements of a BSC.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 11 (CILF S11): Daily Calibration of the Flow Cytometer When a functional assay uses flow cytometric analysis, the following checks shall be performed and documented on each day of use, after maintenance procedures, and after the resolution of any instrumentation failures: a) calibration with stable beads labeled with fluorochromes; b) compensation for spectral overlap for each fluorescent dye that is used for testing; c) determination of adequate fluorescent resolution so that there is a measurable difference between the autofluorescence/non-specific peak and a dimly positive fluorescent peak for each fluorescent parameter used for testing; and, d) standardization to ensure that performance is consistent from day to day.	The manufacturer's recommended procedures should be followed. b) Electronic compensation can be first adjusted with individually fluorescent-labeled beads. Fine tune adjustments should be completed using cells stained with mutually exclusive antibodies brightly labeled with fluorescent dyes. c) Each laboratory should establish acceptable separation between fluorescent peaks of different intensities. Monitoring of acceptable differences between beads with different fluorochrome intensities ensures that the laser and photomultiplier tubes (PMT's) are functioning in a consistent manner. d) The instrument is monitored using a stable fluorescent bead by either using a fixed voltage and measuring the variability of fluorescent peak channel or adjusting voltages to place the fluorescent bead signal at the same peak channel and recording the voltage variability.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 12 (CILF S12): Flow Cytometer Linearity When a functional assay includes quantitative measurement of a biomarker, which is dependent on Mean Fluorescence Intensity (MFI), the instrument shall be assessed for linearity on a monthly basis. The assessment of flow cytometers for linearity shall include: a) linearity at the settings used for clinical measurement using multi-level fluorescent beads; b) fluorescence sensitivity and resolution at settings used for clinical measurement; and, c) evaluation of any photomultiplier tube (PMT) changes.	For accurate quantification of any marker by flow cytometry, it is necessary to ensure fluorescence linearity for all fluorochromes routinely used by the laboratory. a) Correlation coefficient analysis of the Mean Fluorescence Intensity (MFI) versus fluorescent molecules per bead should be equal to or greater than 0.98 (1.0 is the ideal). b) Monitoring assesses the photomultiplier tube (PMT's) range of measurement related to the marker intensity (antigen density on or in the cell) and the ability to resolve populations of different intensities. c) Monitoring provides PMT performance history and large shifts or fluctuations indicate that maintenance may be required.

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Standard	Guidance
Cellular Immunology – Leukocyte Function Sustaining Standard of Practice 13 (CILF S13): Reference Range Requirements The laboratory shall have laboratory-derived reference ranges for each leukocyte function assay; which are: a) divided into pediatric, adult, and geriatric ranges and determined using specimens that are within the range of patient ages of the specimens routinely tested by the laboratory; b) determined using a minimum of 25 known healthy donors for each reference range group (pediatric, adult and geriatric); i. donor demographic records shall include age and sex; ii. these ranges shall be compared to published ranges to verify expected performance c) revised with each assay modification; and d) included on the patient report along with the implementation date of the current reference range.	The Cellular Immunology Submission Guidelines for Assay Approval (CLEP webpage under Test Approval) provides guidance for specimen number and requirements. Assay modification and validation requires submission and approval by the Department of Health. b) If normal subjects within the pediatric or geriatric population group are not available for reference range development, peer-reviewed published ranges from prominent, acknowledged sources will be accepted when the testing methodologies are similar. Reference ranges should include race whenever possible. d) All reporting requirements (including normal values) noted in Section 58-1.11 of 10 NYCRR are applicable. The source of the ranges must be documented in the SOP and cited on reports.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 14 (CILF S14): Normal Control Requirements A freshly prepared whole blood specimen, or a fraction thereof (e.g., peripheral blood mononuclear cells (PBMC) for function), from a healthy donor shall be included as a normal control: a) on each assay plate or analytical run for all Leukocyte Functional assays; b) set up to include stimulated and unstimulated conditions for each analytical run; c) collected in the same anticoagulant as the patient specimen or the anticoagulant used must have been shown to be functionally equivalent during the assay validation; and d) be stored under conditions as similar as possible to those of the test specimens.	c) Validation studies for each anticoagulant must be submitted to the department for NYS approval as required by Validation S5: Performance Specifications.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 15 (CILF S15): Specimen Replicates for Functional Analysis Well or testing replicates should have a coefficient of variation not greater than 20 percent. Greater differences require investigation for procedural errors prior to reporting. a) Functional assays shall be minimally analyzed in duplicate for each condition for each specimen. b) Proliferation and cytolytic assays set up in tissue culture plates shall be analyzed in triplicate for each condition for each specimen.	b) Specimens with abnormally low cell counts may be set up in duplicate for each condition for each specimen. b) Two wells of three may be averaged to report a response, if one of the three wells causes the replicate CVs to be greater than 20 percent. The remaining two wells must be not greater than 20 percent to be reportable.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 16 (CILF 16): Stimulant Concentrations For functional assays, a minimum of two concentrations of stimulant determined to be in the optimal range during validation shall be used per specimen for each analytical run.	Specimens with abnormally low cell counts may be set up with one concentration of stimulant per specimen for each analytical run that was determined to be the optimal during validation.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 17 (CILF S17): Function Assessment Each patient specimen shall be set up for each analytical run to include stimulated and unstimulated conditions.	The optimal storage condition and life span of functional stimulants should be determined during the validation studies. Cells being assessed for functional activity should be characterized and reported whenever possible.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 18 (CILF S18): Evaluation during Functional Peak For *in vitro* functional assays, the laboratory shall examine and test each functional activity during the validated peak activity interval.	

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 19 (CILF S19): Function Quality Control- Normal Specimen Quality control for functional assays shall include: a) evaluation of the stimulated and unstimulated values of the normal control blood analyzed with the patient's specimen(s) for each analytical run. These values shall be determined, documented, and compared to the laboratory-derived reference ranges for each assay analyzed. b) Out of range results for the normal control shall require investigation for procedural error(s) during the assay run and be documented as a non-conformance. c) All patient results within the assay run shall be withheld until technical errors have been ruled out.	b) Requirements for the investigations of non-conformances are described in **Process Review Sustaining Standard of Practice 4** and **Control of Non-Conformities Sustaining Standard of Practice 1**.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 20 (CILF S20): Function Quality Control- Negative, Positive, and Multi-level Controls Negative and positive controls for each functional assay shall be used, whenever available, for each analytic run or plate. Controls demonstrating multiple levels of function shall also be used, when available that demonstrate stability of the functional level over time, for each analytic run or plate. The limit of acceptable day-to-day variance observed with the control cells shall be defined in the procedure manual. If a control demonstrates inappropriate function activity, the analytical run shall be withheld until technical errors have been ruled out.	When available, controls demonstrating multiple levels of function, that demonstrate stability of the functional level over time, are recommended to be used for each analytic run or plate.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 21 (CILF S21): Background Activity Assessments Media, diluent, or carrier solutions cultured with each specimen without the assay stimulant shall: a) be tested during each analytical run; b) demonstrate the lack of function activity; and, c) when functional activity is noted for the unstimulated condition, the analytical run shall be withheld until technical errors have been ruled out.	
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 22 (CILF S22): Verification of Target Cell Labeling Cellular labeling shall exhibit a low spontaneous release of label without effector interactions. Label maintenance shall be monitored and documented for each analytical run.	Assays using target labeling include Cytolytic and Phagocytosis.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 23 (CILF S23): Effector to Target Cellular Ratios Assays using effector to target cell interactions shall be set-up minimally using three cellular ratios between effectors and targets determined to be in the optimal range for the functional activity for each analytical run.	Assays using effector to target ratios include Alloantigen, Cytolytic, and Phagocytosis. Less than three effector to target cell interactions may be used, if appropriately validated and approved by NYS.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 24 (CILF S24): Result Review Results for each test and information used to generate those results shall be reviewed by an individual holding a Certificate of Qualification (CQ) in Cellular Immunology - Leukocyte Function. When a CQ holder is not available: a) a person qualified as a Cellular Immunology- trained supervisor may review and report results during a temporary absence of the CQ holder; b) the review must be in accordance to a protocol approved by the CQ holder prior to his/her absence; c) supervisor-reviewed results shall be reviewed by the CQ holder upon his or her return in a timely manner, not to exceed the length of the absence and this review must be documented.	Information used to generate results may include, but is not limited to, raw data, worksheets, instrument readings, and personal observations. Temporary absence, as defined in Part 58-1 of 10NYCRR, is less than 21 days in duration and includes the inability to access and review the stated above information.

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Standard	Guidance
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 25 (CILF S25): Proliferation Reporting For proliferation assays, results shall be reported as responsive or non-responsive.	For mitogen induced proliferation, the normal response to mitogen is expected to induce a positive stimulation response. For negative response-type antigen-induced proliferation assays, the normal response is the absence of proliferation due to lack of previous exposure. A responsive result is delineated by a proliferative response higher than the established response of unexposed normal donors and/or the stimulation index (response to antigen divided by background response) is not within one or two standard deviations of the mean value for the healthy unexposed control population. For positive response-type antigen-induced proliferation assays, the challenge with an antigen is expected to normally induce a positive stimulation response due to a previous exposure. Attempts should be made to know the vaccine history of the patient so that accurate interpretations can be made. For alloantigen-stimulated proliferation assays (one-way mixed lymphocyte compatibility), the challenge assesses the patient's ability to distinguish self from non-self and is expected to normally induce a positive stimulation response. Testing in this category does not include assays used for tissue typing compatibility.
Cellular Immunology - Leukocyte Function Sustaining Standard of Practice 26 (CILF S26): Reporting Flow Cytometric Results for Functional Analysis Laboratories using flow cytometric analysis of function shall report: a) the characterized population analyzed; and, changes in biomarker expression due to stimulation including percentage and the quantitative change in mean fluorescence intensity (MFI).	

Cellular Immunology

Standard	Guidance
Non-Malignant Immunophenotyping	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 1 (CINM S1):Client Instructions for Specimen Collection and Transport The laboratory shall provide specimen collection and transport instructions to clients indicating: a) the anticoagulant(s) that must be used for each assay offered; b) the maximum allowable transport time so that the laboratory can test within the required assay time frames; c) the requirement for specimen collection date and time needs to be documented; d) blood samples, for other than CD34 Stem Cell analysis, must be handled and transported in a manner to assist in the maintenance of the specimen between 18 - 25 degrees Celsius. Samples for CD34 Stem Cell analysis must be handled and transported in a manner to assist in the maintenance of the specimen between 2 – 8 degrees Celsius; e) blood specimens that do not meet collection and transport criteria will be rejected; and f) any other information considered significant for specimen analysis.	b) Assay time frames are the interval from the time of collection to the time of processing, when the specimen is stained, lysed, and fixed. If the specimen is not fixed during the staining process, then the specimen age end point would be time of data acquisition on the flow cytometer e) Blood specimens that do not meet collection and transport criteria for blood cannot be tested and reported with a disclaimer. These specimens are not irreplaceable. If other specimen types are to be tested, refer to the Client Instructions Standard in the Cellular Immunology- Malignant Leukocyte Immunophenotyping standards. f) For example, transfusion history is needed for glycosylphosphatidylinosistol (GPI)-anchored proteins for paroxysmal nocturnal hemoglobinuria (PNH***)*** analysis.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 2 (CINM S2): Specimen Age for Whole Blood For non-malignant leukocyte immunophenotyping in whole blood, laboratories must: a) process specimens within the manufacturer's recommendations for maximum specimen age; b) when there are no manufacturer's age requirements or unless other time frames are noted in this document, process specimens from the time of collection within: i. 30 hours if using EDTA anticoagulant; ii. 48 hours if using ACD or heparin anticoagulant; or iii. establish the maximum acceptable age of the specimen by internal validation.	The age of the specimen is calculated from the time the specimen is collected to the time the sample is fixed during the staining process, or a non-fixed sample has undergone data acquisition on the flow cytometer. b) Validation studies must be submitted to NYS as required by the Validation Standards in Part 1 – General Systems. For additional information, see the Test Approval webpage for Cellular Immunology. b) Viability analysis (CI-NML.S3) will be required if testing beyond the stated timeframes in i) and ii).
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 3 (CINM S3): Specimen Viability Any specimen tested beyond 30 hours, if using EDTA anticoagulant, and 48 hours, if using ACD or heparin anticoagulant, requires viability analysis. a) Specimens that are less than 50 percent viability must be rejected and a replacement specimen shall be requested; b) Specimens that have less than 80 percent viability may be tested and reported. Results shall be accompanied by a statement indicating that the results are based on a sample that was partially compromised due to the presence of greater than 20 percent non-viable leukocytes.	If the blood specimen is collected into a tube containing a preservative (e.g., Streck Cyto-Chex BCT), viability is not required.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 4 (CINM S4):Antibody Lot Assessments For laboratory developed assays the laboratory must adjust the expected mean fluorescent intensity (MFI) values with each new lot of immunophenotyping antibody reagent for each population analyzed when the MFI changes by more than 15 percent.	Saturation should be determined by titering the antibody to obtain the best signal-to noise-ratio (S/N) for cellular analysis. When MFI differences are greater than 15 percent, the antibody reagent should be re-titered to check signal to noise (S/N) and investigate if the change is related to the lot's fluorochrome to protein (F/P) ratio.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 5 (CINM S5): Antibody Fluorochrome Stability Instructions for the protection of staining reagents from light shall be written in the SOP and include room lighting conditions during the staining process and storage of the stained tube until data acquisition on the flow cytometer. When reagents with different stabilities are combined, the shortest stability length shall be used for the combined reagents. The stability length of each reagent shall be: a) the manufacturer's recommendation under the prescribed room lighting condition; or, b) determined by validation studies if not defined by manufacturer or when light conditions do not conform to manufacturer's recommendations. i. The validation shall determine the maximum incubation or storage length under specific lighting conditions. The maximum allowable difference of the gated population of interest from time zero is a change of plus or minus three percent and a change in the mean fluorescent intensity (MFI) value of 15% or less using specimens from both normal and abnormal individuals.	Some tandem fluorochrome conjugates have short stability periods (e.g., six hours). The stability length is measured from aliquoting the reagent into the staining tube to data acquisition on the flow cytometer. Fluorochromes are sensitive to photobleaching (room lighting conditions) and/or undergo emission spectra changes by prolong exposure to paraformaldehyde (formaldehyde).

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 6 (CINM S6): Daily Calibration of the Flow Cytometer On each day of use, after maintenance procedures, and after the resolution of any instrumentation failures, the following checks shall be performed on the flow cytometer and documented: a) calibration with stable beads labeled with fluorochromes; b) compensation for color spectral overlap for each fluorescent dye that is used for testing; c) determination of adequate fluorescent resolution so that there is a measurable difference between the autofluorescence/non-specific peak and a dimly positive fluorescent peak for each fluorescent parameter used for testing; and, d) standardization to ensure that performance is consistent from day to day.	b) Electronic compensation can be first adjusted with individually fluorescent-labeled beads. Fine tune adjustments should be completed using cells stained with mutually exclusive antibodies brightly labeled with fluorescent dyes. c) Each laboratory should establish acceptable separation between fluorescent peaks of different intensities. Monitoring of acceptable differences between beads with different fluorochrome intensities ensures that the laser and photomultiplier tubes (PMT's) are functioning in a consistent manner. d) The instrument is monitored using a stable fluorescent bead by either using a fixed voltage and measuring the variability of fluorescent peak channel or adjusting voltages to place the fluorescent bead signal at the same peak channel and recording the voltage variability.

Cellular Immunology

Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 7 (CINM S7): Flow Cytometer Linearity Flow cytometers shall be assessed for linearity on a monthly basis and documented. The assessment of flow cytometers for linearity shall include: a) linearity at the settings used for clinical measurement using multi-level fluorescent beads. Correlation coefficient analysis of the Mean Fluorescent Intensity (MFI) versus fluorescent molecules per bead must be equal to or greater than 0.98 (1.0 is the ideal); b) Fluorochrome sensitivity and resolution at settings used for clinical measurement; and c) evaluation of any photomultiplier tube (PMT) changes.	All markers used in the analysis must be FDA cleared or approved to be exempt from monthly linearity checks**.** For accurate quantification of any marker by flow cytometry, it is necessary to ensure fluorescence linearity for all fluorochromes routinely used by the laboratory. b) Monitoring assesses the photomultiplier tube's (PMT's) range of measurement related to the marker intensity (antigen density on or in the cell) and the ability to resolve populations of different intensities. c) Monitoring provides photomultiplier tube (PMT) performance history and large shifts or fluctuations indicate that maintenance may be required.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 8 (CINM S8):Single Platform Volume Delivery Accuracy When using single-platform methods that are dependent on accurate volume delivery, the laboratory shall verify the accuracy of the volume delivery by automated or manual volume delivery apparatus used for the blood specimen and/or bead delivery, monthly using a gravimetric method, control beads, or another appropriate procedure.	If volume inconsistencies are noted or a manual pipette has been dropped, the volume dispensing apparatus must be checked immediately before resuming patient testing, as required in the Facility Design and Resource Management (FDRM) standards.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 9 (CINM S9):Single Platform Requirements When the laboratory uses single-platform methods that use fluorescent bead counts, a) the manual shall indicate the use of a lyse/no wash procedure; and, b) bead event collection shall be 1000 or greater per sample tube.	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 10 (CINM S10): Normal Reference Range Requirements Except for stem cell analysis, the laboratory shall verify or establish reference ranges for each leukocyte immunophenotyping marker. The laboratory shall have laboratory-derived reference ranges for each assay; a) which are:divided into pediatric, adult, and geriatric ranges and determined using specimens that are within the range of patient ages of the specimens routinely tested by the laboratory; b) determined using a minimum of 25 known healthy donors for each reference range group (pediatric, adult and geriatric) i. donor demographic records shall include age and sex; ii. these ranges shall be compared to published ranges to verify expected performance c) revised with each assay modification; and, d) included on the patient report along with the implementation date of the current reference range.	The Cellular Immunology Submission Guidelines for Assay Approval (CLEP webpage under Test Approval) provides guidance for specimen number and requirements. Assay modification and validation requires submission and approval by the Department of Health. a) There are FDA cleared or approved test systems that require the development of reference ranges by the laboratory. b) If normal subjects within the pediatric or geriatric population group are not available for reference range development, peer-reviewed published ranges from prominent, acknowledged sources will be accepted when the testing methodologies are similar or equivalent. Reference ranges should include race whenever possible. When published references for a specific analyte do not describe the requirement to define separate age populations, a single reference group is acceptable. d) All reporting requirements (including normal values) noted in Section 58-1.11 of 10 NYCRR are applicable. The source of the ranges must be documented in the SOP and cited on reports.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 11 (CINM S11): Control Requirements For each non-malignant leukocyte immunophenotyping marker being assayed, control(s) must be used on each day of testing using: a) two levels of whole blood equivalent commercial controls when available that fall within two different areas of the reportable range; b) one commercial and a freshly prepared whole blood specimen from a healthy donor, when the commercial control levels are not significantly differently; or c) a freshly prepared whole blood specimen from a healthy donor when commercial controls are not available. i. When a fresh whole blood control is used, it must be collected in the same anticoagulant as the patient specimen, or the anticoagulant used must have been validated to produce equivalent immunophenotypic results.	Equivalent commercial control should have same matrices as the expected patient specimen to allow the complete testing process to be quality controlled. The only allowed addition is preservative. The laboratory must verify the manufacturer's ranges for each lot of the commercial controls as required in the Examination Procedure (EP) standards. If sample preparation problems occur, additional control specimens should be used for trouble-shooting purposes. The whole blood normal control for each assay shall be stored under conditions as similar as possible to those of the test specimens.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 12 (CINM S12): Positive-Negative Bio-marker Determination For laboratory developed assays, the laboratory shall: a) use negative staining cells within the same gated population, when using SS/CD45 gating, to determine the demarcation between positive and negative regions for isotype-matched antibodies; or, b) use isotype control antibodies for setting analysis cursors that distinguish negative from positive staining cells when using Forward Scatter/ Side Scatter gating techniques and/or analyzing cellular antigens of dim fluorescent intensity. These immunophenotyping negative controls (isotype controls) shall be isotype matched antibody at similar concentrations and fluorochrome to protein (F/P) ratios as the test antibody.	a) Biomarkers within the patient's testing panel that have concise negative and positive staining patterns may be used to define negative staining in other panel tubes that may contain more diffuse staining when analyzing within the same gated population with antibodies that are isotype-matched. The isotype control is the negative control to detect non-specific antibody binding.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 13 (CINM S13):Listmode Data Storage The information pertaining to all leukocyte populations (no restrictive population gate) shall be stored using list-mode format for a minimum of two months	Histograms must be saved for two years as required in Part 58 and Part 1 General Systems Standards.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 14 (CINM S4): Result Review Results for each test and information used to generate those results shall be reviewed by an individual holding a certificate of qualification (CQ) in Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping prior to reporting. When a CQ holder is not available: a) a person qualified as a Cellular Immunology- trained supervisor may review and report results during a temporary absence of the CQ holder; b) the review must be in accordance to a protocol approved by the CQ holder prior to his/her absence; and c) supervisor reviewed results shall be reviewed by the CQ holder upon his or her return in a timely manner, not to exceed the length of the absence, and this review must be documented.	Information used to generate results may include, but is not limited to, raw data, worksheets, instrument readings, and personal observations. Minimally, the flow cytometric print-outs should be reviewed for accuracy in population gating and marker analysis. Temporary absence, as defined in Part 58-1 of 10NYCRR, is less than 21 days in duration and includes the inability to access and review the stated above information.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 15 (CINM S15): Lymphocyte enumeration- Lymphocyte Event Collection At least 2,500 lymphocytes shall be collected per sample tube for quantification. When the required number of lymphocytes cannot be collected, the number shall be stated on the patient report with a disclaimer that the optimal collection of events is 2,500 lymphocytes.	In severe late stage AIDS or immunosuppressed patients, cell counts may be very low; under these conditions, collection of less than 2,500 lymphocytes is acceptable.

Cellular Immunology	
Standard	**Guidance**
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 16 (CINM S16): Lymphocyte enumeration- Multi-color CD Subset Definitions	**STANDARD DELETED effective June 1, 2017**
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 17 (CINM S17): Lymphocyte enumeration- Quality Control using CD3 Tube Replicate The CD3 replicate shall be monitored when the analysis panel contains more than one stained tube. a) The CD3 percentage values shall not exceed a difference of 3 between the highest and lowest CD3 value within the patient's stained panel tubes; and b) The CD3 absolute values shall not differ by greater than 10 percent among the CD3 absolute values within the patient's stained panel tubes.	The use of different fluorochromes or different monoclonal antibodies for CD3 could affect this determination. When CD3 values do not replicate, the laboratory should document that the specimen was repeated and/or restained. b) Specimens with abnormally low CD3 cell counts may require a greater allowable difference between replicate tubes within the patient's stained panel, but should not exceed 20 percent.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 18 (CINM S18): Lymphocyte enumeration- Quality Control using Lymphosum Determination When a lab analyzes T, B and NK cells, the lymphosum must be within 90 -105 percent.	Lymphosum refers to the sum of all subsets of lymphocytes ($CD3^+$ plus $CD19^+$ plus $CD3^-/CD56^+$ and/or $CD16^+$ cells). The laboratory should troubleshoot for technical difficulties when the lymphosum is out of optimal range.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 19 (CINM S19): Lymphocyte enumeration- Quality Control using T-Sum Determination The T-sum shall be monitored as follows: a) for three color analysis : The sum of $CD3^+CD4^+$ and $CD3^+CD8^+$ must be within 10 percent of the total CD3 mean. b) for four or more color analysis: The summation of the single positive T cells ($CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$ cells), the double positive T cells ($CD3^+CD4^+CD8^+$) and the double negative T cells ($CD3^+CD4^-CD8^-$) shall not exceed a difference of 3 of the total CD3 percentage mean.	If a greater difference is found, the laboratory should repeat the analysis, including restaining, to confirm that no preparation problems occurred. a) Three color analysis: A greater variance is acceptable in patients with an increased population of $CD4^-CD8^-CD3^+$ cells (e.g., delta/gamma T cells). b) As required in these standards (Cellular Immunology Non-Malignant Leukocyte Immunophenotyping Standard 16), i. T cell subsets need to be assessed for single-positive, double-positive, and double-negative expression of CD4 and CD8 subsets of CD3 lymphocytes. ii. Double positive (DP) $CD4^+CD8^+$ T lymphocytes should not be included in the single positive T cell populations and will affect the T-sum determination if this DP T cell subset has not been resolved.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 20 (CINM S20):Lymphocyte Enumeration- Reporting Requirements Unless the single platform instrumentation only provides absolute numbers, percentages and absolute numbers of lymphocyte subsets shall be reported.	
CD34 Stem Cell Enumeration	*All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed.*

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 21 (CINM S21): CD34 Stem Cell Enumeration- Specimen Age For CD34 stem cell enumeration, laboratories shall process specimens within the following age limits: a) the manufacturer's recommendations for maximum specimen age cut-offs for the specimen type and the assay testing system used; or b) for laboratory developed assays, specimens must be tested within 24 hr using timeframes validated by the laboratory and approved by NYS that demonstrates results at time zero (within zero to four hours of collection) and at the maximum time do not exceed a difference of 3 percent.	The age of a specimen is the interval from the time of collection to data acquisition. Data acquisition is the process of acquiring event information, including light scatter and fluorescent parameters, from the stained specimen tube on the flow cytometer. The specimen needs to be handled and transported in a manner that maintains an optimal viable condition and expedites testing.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 22 (CINM S22): CD34 Stem Cell Enumeration- Apheresis Specimen Requirements On apheresis specimens, the laboratory shall quantify the number of cells prior to staining. If a dilution is necessary to be within the test system's target cell range: a) the dilution buffer shall contain a support protein to reduce or eliminate the "vanishing bead" phenomena, when using single platform methodologies; and, b) the dilution factor shall be documented and used for cell count calculations.	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 23 (CINM S23): CD34 Stem Cell Enumeration- Specimen Staining/Processing Reagent Requirements For CD34 stem cell enumeration, the laboratory shall: a) use CD34 antibody reagent that: i. bind CD34 class II or class III epitopes; ii. be conjugated with a bright fluorochrome (eg., PE); and iii. not include the use of FITC conjugated antibodies to CD34 class II; b) use CD45 antibody reagent that detects all isoforms and glycoforms; and c) use ammonium chloride lyse reagent without fixative in a lyse/no wash manner.	

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Standard	**Guidance**
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 24 (CINM S24): CD34 Stem Cell Enumeration- Viable Cell Assessment CD34 stem cell enumeration shall include the simultaneous determination of viable cells.	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 25 (CINM S25): CD34 Stem Cell Enumeration- Analysis Requirements When performing CD34 stem cell analysis, the laboratory shall: a) collect at least 100 stem cell events per sample for quantification; and b) collect at least 1,000 bead events, if single platform methodologies are used.	Collection of 75,000 leukocyte events is strongly recommended. Sequential Boolean gating strategy is strongly recommended with a threshold/discriminator to remove non-leukocytes events (debris) in the plot determining the leukocyte gate. The gating strategy should exclude dead cells, platelet aggregates, myeloid/monocytic cells from the final determination while identifying stem cells based on bright $CD34^{+}$, dim to intermediate CD45 staining, and low side scatter (granularity).
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 26 (CINM S26):CD34 Stem Cell Enumeration- Control Requirements For the enumeration of CD34 stem cells, a low and high level control shall be assessed on each day of testing.	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 27 (CINM S27):CD34 Stem Cell Enumeration- reporting requirements Viable CD34 stem cells shall be quantified and reported as absolute number of viable cells per microliter.	
Analysis of GPI anchored proteins for PNH diagnosis	*All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed.*

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 28 (CINM S28): Analysis of GPI-anchored proteins for PNH diagnosis – Specimen Stability Specimens for glycosylphosphatidylinosistol (GPI) anchorage of Paroxysmal Nocturnal Hemoglobinuria (PNH) immunophenotyping shall: a) be processed within 48 hours of collection, when fixatives are used during the staining process; or b) undergo data acquisition on the flow cytometer within 48 hours of collection, when fixatives are not used in the staining process.	
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 29 (CINM S29): Analysis of GPI-anchored proteins for PNH diagnosis - Analysis Requirements A normal blood shall be stained and analyzed concurrently with patient specimen to define normal expression in the analysis of GPI anchored antigens	Both specimens (normal and patient) should be collected within four hours of each other since antigens are naturally shed post-collection. The degree of antigen expression determines the severity of the disease. Variable composite phenotype expressions may be present for each cell lineage within an abnormal specimen. Use of antibody and/or non-antibody controls is also recommended to define non-specific binding as the specimen ages post collection.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 30 (CINM S30): Analysis of GPI anchored proteins for PNH Diagnosis - Positive Staining Control Analysis for Paroxysmal Nocturnal Hemoglobinuria (PNH) Diagnosis shall include a marker for a transmembrane antigen in each staining tube to provide a positive staining antibody control. Staining quality (percentage and mean fluorescent intensity) shall be documented.	Use of a lineage marker for this purpose can also provide assistance for population identification and gating (eg., for WBC's- CD45, CD15, and/or CD64; for RBC's- anti-glycophorin A or CD235a).

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 31 (CINM S31): Analysis of GPI anchored proteins for PNH Diagnosis - Requirements for Routine Analysis Routine analysis for GPI-anchorage (PNH) shall be used for the detection of specimens containing 1% or greater events of the anchorage deficiency within a gated population. For routine GPI anchored antigen (PNH) analysis, a minimum of 5,000 events shall be collected for each population analyzed.	A low differential for the monocyte population may permit fewer events to be collected for this population. Two parameter density plots of both GPI markers should be used to determine the double negative events to define complete deficiency (Type III) and partial deficiency (Type II) to be defined by the events of intermediate brightness for both markers.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 32 (CINM S32): Analysis of GPI anchored proteins for PNH Diagnosis - Requirements for High Sensitivity Analysis High sensitivity analysis for GPI-anchorage (PNH) shall be used for the detection of specimens containing less than 1 to 0.01 percent of events demonstrating anchorage deficiency within a gated population. High sensitivity analysis for GPI-anchorage (PNH) immunophenotyping shall: a) collect a minimum of 250,000 events for each population analyzed; b) use two parameter density plots of both GPI markers to determine the double negative events to define complete deficiency (Type III) and partial deficiency (Type II) to be defined by the events of intermediate brightness for both markers.	A low differential for the monocyte population may permit fewer events to be collected for this population. High sensitivity analysis for GPI-anchorage (PNH) should be analyzed with a minimum of two lineage transmembrane biomarkers with light scatter parameters to accurately define each leukocyte population analyzed using a sequential Boolean strategy; Precautions should be taken to reduce cell carryover between analyzed marker tubes during rare event analysis.

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Standard	Guidance
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 33 (CINM S33): Analysis of GPI anchored proteins for PNH Diagnosis - Review Criteria and Reporting The laboratory shall review the patient's history of any recent blood transfusions when assessing GPI anchored antigens. If laboratory cannot obtain transfusion history, a disclaimer must be on the report. The total PNH content per cell population shall be reported while acknowledging complete (type III) and partial (type II) deficiency components. Disease confirmation shall require an expression deficiency of at least two different GPI biomarkers including monoclonal antibodies and/or FLAER directed against two different GPI-anchored antigens assessed on a minimum of two different cell lineages (*e.g.*, RBC and neutrophils).	Transfused donor cells will dilute the patient's blood phenotype composition. FLAER is a fluorescently labeled inactive variant of the protein aerolysin that selectively binds GPI anchors on leukocytes. FLAER must be analyzed with an additional GPI biomarker per leukocyte lineage.
Leukocyte Adhesion Deficiency (unstimulated)	*All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed. Laboratories that assess the stimulate upregulation using cell culture methods fall under the category of Cellular Immunology – Leukocyte Function.*
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 34 (CINM S34): Leukocyte Adhesion Deficiency (unstimulated expression) – Event Collection A minimum of 5,000 events shall be collected per population analyzed.	

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Standard	**Guidance**
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 35 (CINM S36): Leukocyte Adhesion Deficiency (unstimulated expression) – Positive Staining Control Analysis for Leukocyte Adhesion Deficiency (LAD) shall include CD45, or a lineage-specific CD marker, in each staining tube to provide a positive staining antibody control. Staining quality (percentage and mean fluorescence intensity) shall be documented.	These markers should be used for leukocyte population identification for gating. Analysis for LAD Type 1 should include markers for the leukocyte β2 integrins (staining panels of CD18 with CD11a and CD11b. Analysis for LAD Type 2 should include the CD15s marker.
Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 36 (CINM S36): Leukocyte Adhesion Deficiency (unstimulated expression)- Report Requirements The patient report shall include leukocyte population identification with the biomarker expressions including percentage and the quality of expression or Mean Fluorescence Intensity (MFI).	

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Standard	Guidance
Malignant Leukocyte Immunophenotyping	
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 1 (CIML S1): Client Instructions for Specimen Collection and Transport The laboratory shall provide specimen collection and transport instructions to clients indicating: a) the anticoagulant(s), saline, or medium that must be used for each type of specimen to be collected; b) the requirement for specimen collection date and time must be documented with each specimen; c) if testing will occur in less than eight hours, specimens must be handled and transported in a manner to assist in the maintenance of the specimens between 18 and 25 degrees Celsius prior to testing. If testing is expected to occur in greater than eight hours, specimens must be handled and transported with cold packs to maintain specimens between 2 and 8 degrees Celsius prior to testing; d) the maximum time between collection and receipt so that the laboratory can test within required assay time frames; and, e) any other information considered significant for specimen analysis.	d) Assay time frames are the interval from the time of collection to the time of processing, when the specimen is stained, lysed, and fixed. If the specimen is not fixed during the staining process, then the specimen age end point would be time of data acquisition on the flow cytometer

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 2 (CIML S2): Specimen Age and Integrity Leukemia/lymphoma specimens: a) shall be tested within 48 hours of collection unless the specimen is deemed irreplaceable and specimen re-collection would be harmful to the patient; and b) shall be visually inspected for evidence of deterioration or unacceptable conditions upon receipt. Specimens, which are fixed, frozen, warmer than 37 degree Celsius or, in the case of peripheral blood or bone marrow, clotted or hemolyzed, shall be rejected.	Leukemia/Lymphoma specimens should be handled and transported in a manner that maintains optimal condition and expedites testing. Some specimen types and/or malignancies are more fragile and should be tested earlier.
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 3 (CIML S3): Specimen Viability Leukemia/lymphoma specimens shall be assessed for viability during specimen processing, prior to staining and fixation, on a non-fixed aliquot of the specimen's single cell suspension. a) Blood specimens greater than eight hours post collection shall be assessed for viability. Blood specimens that are less than 50 percent viability must be rejected and a replacement specimen shall be requested; b) When specimens have less than 80 percent viability, the report must note that the results are based on a sample that was partially compromised due to the presence of greater than 20% non-viable leukocytes; c) Specimens having less than 50 percent viability shall be reported only when it is an irreplaceable sample and an abnormal population is definitively determined by the combination of the flow cytometry results with other clinical and technical features of the case.	In some cases (e.g., CSF), an extremely low cell count may not allow viability to be analyzed. For such cases, the report must note that a viability assessment could not be determined due to the very low cell count. c) Examples of irreplaceable specimens are thymus, lymph node, spleen, and bone marrow.

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Standard	**Guidance**
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 4 (CIML S4: Leukocyte Count Adjustment Prior to staining, the laboratory shall adjust the white blood cell concentration to optimize cell-to-reagent ratio and instrument acquisition event rate. The initial leukocyte concentration, the dilution calculations, and final leukocyte concentration for the specimen shall be documented.	
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 5 (CIML S5): Antibody Lot Assessments For each new lot of immunophenotyping antibody reagent, the laboratory must adjust the expected mean fluorescent intensity (MFI) values for each population analyzed when the MFI changes by more than 20 percent.	Lot checks should be conducted using a normal control blood to make the additional comparison against the laboratory's determination of expected normal expression levels. Saturation should be determined by titering the antibody to obtain the best signal-to noise (S/N) ratio for cellular analysis. When MFI differences are over 20 percent, the antibody reagent should be re-titered to check S/N and investigated if the change is related to the lot's fluorochrome to protein (F/P) ratio.

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Standard	Guidance
Cellular Immunology –Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 6 (CIML S6): Antibody Fluorochrome Stability Instructions for the protection of staining reagents from light shall be written in the SOP and include room lighting conditions during the staining process and storage of the stained tube until data acquisition on the flow cytometer. When reagents with different stabilities are combined, the shortest stability length shall be used for the combined reagent. The stability length of each reagent shall be: a) the manufacturer's recommendations under the prescribed room lighting condition; or b) determined by validation studies if not defined by manufacturer or when light conditions do not conform to manufacturer's recommendations. i. The validation shall determine the maximum incubation or storage length under specific lighting conditions. The maximum allowable difference from time zero, within the gated population, is a percent change of plus or minus three and a change in the mean fluorescent intensity (MFI) value of 20% or less using specimens from both normal and abnormal individuals.	Some tandem fluorochrome conjugates have short stability periods (e.g. six hours). The stability length is measured from aliquoting the reagent into the staining tube to data acquisition on the flow cytometer. Fluorochromes are sensitive to photobleaching (room lighting conditions) and/or undergo emission spectra changes by prolong exposure to paraformaldehyde (formaldehyde).

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Standard	Guidance
Cellular Immunology –Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 7 (CIML S7): Daily Calibration of the Flow Cytometer On each day of use, after maintenance procedures, and after the resolution of any instrumentation failures, the following checks shall be performed on the flow cytometer and documented: a) calibration with stable beads labeled with fluorochromes; b) compensation for color spectral overlap for each fluorescent dye that is used for testing; c) determination of adequate fluorescent resolution so that there is a measurable difference between the autofluorescent/non-specific peak and a dimly positive fluorescent peak for each fluorescent parameter used for testing; and d) standardization to ensure that performance is consistent from day to day.	The manufacturer's recommended procedures should be followed. b) Electronic compensation can be first adjusted with individually fluorescent-labeled beads. Fine tune adjustments should be completed using cells stained with mutually exclusive antibodies brightly labeled with fluorescent dyes. c) Each laboratory should establish acceptable separation between fluorescent peaks of different intensities. Monitoring of acceptable differences between beads with different fluorochrome intensities ensures that the laser and photomultiplier tubes (PMT's) are functioning in a consistent manner. d) The instrument is monitored using a stable fluorescent bead by either using a fixed voltage and measuring the variability of fluorescent peak channel or adjusting voltages to place the fluorescent bead signal at the same peak channel and recording the voltage variability.
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 8 (CIML S8): Flow Cytometer Linearity On a monthly basis, laboratories performing malignant leukocyte immunophenotyping shall assess and document their flow cytometer(s) for fluorescence linearity. The assessment of flow cytometers for linearity shall include: a) linearity at the settings used for clinical measurement using multi-level fluorescent beads. Correlation coefficient analysis of the Mean Fluorescent Intensity (MFI) versus fluorescent molecules per bead must be equal to or greater than 0.98 (1.0 is the ideal); b) fluorescence sensitivity and resolution at settings used for clinical measurement; and c) evaluation of any photomultiplier tubes (PMT) changes.	The intensity of cellular antigens (markers) can assist in assessing the characteristics of the aberrant population(s). For accurate quantification of any marker by flow cytometry, it is necessary to ensure fluorescence linearity for all fluorochromes routinely used by the laboratory. b) Monitoring assesses the PMT's range of measurement related to the marker intensity (antigen density on or in the cell) and the ability to resolve populations of different intensities. c) Monitoring provides photomultiplier tubes (PMT) performance history and large shifts or fluctuations indicate that maintenance may be required.

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 9 (CIML S9): Normal Control Requirements A freshly prepared whole blood specimen from a healthy donor shall be tested as a normal control at least monthly for malignant leukocyte immunophenotyping and results shall be documented. The normal control shall be used to evaluate: a) normal staining expression (percentage and intensity) of the biomarkers on all leukocyte populations; and b) appropriate flow cytometer(s) settings (PMT voltages, color compensation, etc) to achieve optimal resolution of leukocyte subpopulations and biomarker fluorescent quality and resolution.	Cell lines should also be used for the assessment of antibody reagents that are not positive on normal leukocyte populations. The whole blood normal control for each assay should be collected and stored under conditions as similar as possible to the whole blood test specimen. The normal control specimen should be used for the lot check process prior to finishing the current reagent lot to evaluate for lot differences in the laboratory derived normal expression definitions.
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 10 (CIML S10): Positive-Negative Bio-marker Determination To assess biomarker expression demarcation as positive or negative: a) negative staining cells within the same gated population shall be used if the antibodies are isotype-matched; b) when analyzing cellular antigens of dim fluorescent intensity, isotype control antibodies shall be used to assist the setting of cursors that distinguish negative from positive staining cells. These negative controls must be isotype-matched at similar concentrations and fluorochrome to protein (F/P) ratios as the test antibody.	a) Biomarkers within the patient's testing panel that have concise negative and positive staining patterns may be used to define negative staining in other panel tubes that may contain more diffuse staining when analyzing within the same gated population with antibodies that are isotype-matched unless the antibody of interest is polyclonal. The isotype control antibodies are the negative control to detect non-specific antibody binding.

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Standard	**Guidance**
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 11 (CIML S11): Data Acquisition- Event Collection Data acquisition shall minimally include the collection of 10,000 leukocyte events, or 5,000 if the specimen presents as a single population, excluding cellular debris and dead cells. When the required number of leukocyte events cannot be collected, the patient report shall include a disclaimer that the optimal event collection was unattainable due to low cellular content of the specimen.	A specimen with low cellular content may require the collection of fewer events (e.g., cerebral spinal fluid and fine needle aspiration).
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 12 (CIML S12): Data Acquisition- Population Resolution and Gating When performing malignant leukocyte immunophenotyping, the laboratory shall: a) optimize for the best population separation to allow gates or regions to be cleanly drawn on specific leukocyte populations while reducing debris and non-leukocyte contamination; and b) complete data analysis using multiple gated regions set on the apparent populations, including normal leukocyte populations, with minimal contamination from other cell populations.	a) The operator should strive to fully use the plot area while reducing or eliminating any population overlap. Inclusion of cellular debris and dead cell events should be reduced by use of threshold/discriminator settings.

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 13 (CIML S13): Specimen Quality Assurance To ensure testing accuracy prior to reporting, the laboratory must document that: a) replicate antibody use on the aberrant population(s) within the patient's specimen testing panel is consistent (percentage and intensity) when using the same clone, fluorochrome, and manufacturer (conjugation process), and b) a normal leukocyte population within the specimen is analyzed concurrently to ensure normal staining activities, unless the specimen doesn't contain any normal population(s).	a) If results demonstrate inconsistencies with regard to the antigenic profile of the aberrant cell, the laboratory should review the analysis for procedural error and re-stain if necessary. b) The selected normal leukocyte population within the patient sample should be compared to the laboratory-derived normal expression determinations for the population analyzed. If differences are noted, the analysis should be reviewed for technical difficulties.
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 14 (CIML S14): Result Review Results for each test and information used to generate those results shall be reviewed by an individual holding a certificate of qualification (CQ) in Cellular Immunology – Malignant Leukocyte Immunophenotyping prior to release of test results. When a CQ holder is not available: a) a person qualified as a Cellular Immunology- trained supervisor or hematopathologist may review and document results during a temporary absence of the CQ holder; b) this process shall be in accordance with a protocol approved by the CQ holder prior to his/her absence; c) supervisor- or hematopathologist- reviewed results shall be reviewed by the CQ holder upon his or her return in a timely manner, not to exceed the length of the absence, and this review shall be documented.	Information used to generate results may include, but is not limited to, raw data, worksheets, instrument readings, and personal observations. Minimally, the flow cytometric print-outs should be reviewed for accuracy in population gating and biomarker analysis. Temporary absence, as defined in Part 58-1 of 10NYCRR, is less than 21 days in duration and includes the inability to access and review the stated above information. A qualified hematopathologist is licensed physician who is board certified for this subspecialty by the American Board of Pathology.

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 15 (CIML S15): Listmode Data Storage The information pertaining to all viable leukocyte populations (no restrictive population gate) shall be stored using list-mode format for a minimum of three months.	
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 16 (CIML S16): Report Requirements In addition to the report content requirements detailed in the General Systems Standard, the leukemia/lymphoma report shall include: a) specimen viability; b) descriptions of the light scatter characteristics of each identified aberrant population; c) the percentage of each identified aberrant population within the patient specimen; d) biomarker expression levels that are abnormal (higher or lower) for those observed on normal cells of similar hematopoietic lineage. The patient report shall include a description of the quality of staining (e.g., dim or low intensity, bright or high intensity); e) description of lineage and stage for each identified aberrant population; and f) declaration of any specimen condition that was less than optimal.	The flow cytometric results should be correlated with pathology results, when available, and whenever appropriate, should indicate the monoclonality and/or biphenotypic characteristics.

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 17 (CIML S17): Minimal Residual Disease Analysis - Client Instructions In addition to the requirements described in CI-ML.S1, the client instructions for Minimal Residual Disease (MRD) analysis shall also require: a) the submission of the patient's medical history; b) acknowledgement of collection timing during the treatment regimens related to the type of specimen submitted; c) defined amount of specimen (volume or cell number) needed to report test results for rare event analysis; and d) expedited transport to allow specimen age of less than 30 hours.	The standards for routine malignant immunophenotyping also need to be followed, but the more stringent requirement applies. a) The patient's medical history is critical for the testing laboratory to determine effective antibody selections and gating strategy to enrich the collection of rare event aberrant cells. b) The laboratory must capture the rare aberrant event while discriminating from normal mature cells and normal regenerating progenitor cells. Knowledge of the collection timing will assist the testing laboratory to discriminate between normal circulating cells and the minimal residual disease. c) Client instructions should convey assay requirements for specimen volume or cell number required to perform testing. Adequate numbers and viability of the cell type needed for the assay should be known prior to making the requested analysis. d) A fresher specimen will provide the least amount of dead cells comprising the analysis.
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 18 (CIML S18): Minimal Residual Disease Analysis- Event Collection for Rare Event Analysis Event collection for Minimal Residual Disease Analysis shall require: a) the collection of 500,000 leukocyte events per tube. When the required number of leukocyte events cannot be collected, the patient report shall include a disclaimer that the optimal event collection was unattainable; and b) reduction of cell carryover between analysis tubes.	The standards for routine malignant immunophenotyping also need to be followed, but the more stringent requirement applies. a) Client instructions should convey individual assay requirements for specimen volume or cell number required to perform testing. b) Sampling a water tube between the sample tubes can reduce carryover.

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Standard	Guidance
Cellular Immunology – Malignant Leukocyte Immunophenotyping Sustaining Standard of Practice 19 (CIML S19): Minimal Residual Disease Analysis- Antibody Panel Design and Analysis Requirements	
Requirements for Antibody Panel Design and Analysis for Minimal Residual Disease Analysis shall include:	The standards for routine malignant immunophenotyping also need to be followed, but the more stringent requirement applies.
a) the investigation of the patient's medical history to provide assistance in antibody selection and gating strategy to enrich the collection of the patient's rare event aberrant cells. Records of the patient's previous immunophenotypic analysis shall be maintained by the testing laboratory;	
b) design of sufficient antibody panels to enable the detection of new phenotypic profiles caused by immunophenotypic shifts. Tube antibody panels shall be comprised of a minimum of four colors with the use of replicate fluorescent antibodies between the stained tubes within the patient's testing panel;	b) Six or more colors per tube is recommended as more colors per tube will provide increased sensitivity and accuracy. It is also recommended to use of FITC and PE for the most critical aberrancy biomarkers with PE being used for the low expression biomarkers.
c) use of sequential gating to enable the detection of the rare event aberrant cell and discrimination from normal mature cells and normal regenerating progenitor cells. Knowledge of specimen collection timing within the patient's treatment regimen shall provide supportive assistance in this determination and shall be documented by the testing laboratory.	

Cytokines	
Standard	**Guidance**
The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. (CK S1 revised and effective July 14, 2014)	It is recommended that World Health Organization (WHO) international cytokine standards be evaluated as additional inter-assay control when available. Since these assays are not cleared or approved by the FDA, the laboratory must submit copies of package inserts and patient reports before initiating testing as described in the Submission Guidelines. These Guidelines can be found at www.wadsworth.org/clep.
Cytokine Standard 1 (CK S1) Laboratories shall establish or verify the reference interval for each cytokine for each matrix tested.	Normal range of values should be established for each matrix (e.g., serum, plasma, or CSF). The effect of diurnal variation on cytokine levels should be taken into consideration and should be included in collection instructions.
Cytokine Standard 2 (CK S2) All results that fall above the reference interval (highest point on the linear portion of standard curve) shall be diluted and retested.	False positive results may be obtained when the specimen is run neat due to matrix interference.
Cytokine Standard 3 (CK S3) All specimens shall be run in duplicate with non-automated methods unless validation studies indicate acceptable precision.	Laboratories should establish an acceptable range of variation for duplicate values (e.g., less than 20 percent coefficient of variation).