

Cellular Immunology Categories

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>Cellular Immunology Standard of Practice 1 (CI S1): Client Instructions for Specimen Collection</p> <p>In addition to the requirements in Specimen Processing Standard of Practice 1, the laboratory must provide clients with specimen submission instructions including:</p> <ul style="list-style-type: none"> a) acceptable anticoagulant(s), collection containers, and/or preservatives for each assay; b) assays requiring documentation of the specimen collection date and time; c) criteria for longitudinal study of function monitoring; d) for Minimal Residual Disease (MRD) analysis: <ul style="list-style-type: none"> i. submission of the patient’s medical history relevant to the test; ii. specimen volume or cell number needed for rare event analysis; and e) any other information considered significant for specimen analysis. 	<p>For leukocyte function, 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 an in-house derived normal control should assist in controlling for possible shipment effects.</p>
<p>Cellular Immunology Standard of Practice 2 (CI S2): Client Instructions for Specimen Transport and Storage Prior to Analysis</p> <p>In addition to the requirements in Specimen Processing Standard of Practice 1, the laboratory must provide specimen</p>	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clip/clinical-labs/obtain-permit/test-approval.</p>

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>transport instruction to clients, in the absence of manufacturer instructions, indicating requirements for:</p> <ul style="list-style-type: none"> a) specimen transport temperatures of 18-25 degrees Celsius for all specimens, except for: <ul style="list-style-type: none"> i. CD34 stem cell analysis and malignant leukocyte immunophenotyping that must be maintained at 2-8 degrees Celsius when testing will occur greater than eight (8) hours after collection; b) the allowable transport time for each assay, with analysis of specimens for leukocyte function not exceeding twenty-four (24) hours post collection unless the laboratory developed test (LDT) has been approved by the Department; and c) any other information considered significant for specimen transport. 	
<p>Cellular Immunology Standard of Practice 3 (CI S3): Specimen Acceptance and Rejection Criteria</p> <p>In addition to the requirements in Specimen Processing Standard of Practice 4, the laboratory must have a procedure to reject specimens that are not collected according to Cellular Immunology Standards of Practice 1 and 2 and that are:</p> <ul style="list-style-type: none"> a) clotted or hemolyzed; or b) fixed or frozen; or c) transported at greater than 37 degrees Celsius. 	

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>Cellular Immunology Standard of Practice 4 (CI S4): Specimen Viability Testing</p> <p>The lab must perform viability testing prior to testing on all:</p> <ul style="list-style-type: none"> a) leukocyte function specimens and CD34 hematopoietic stem cell samples; b) leukemia/lymphoma specimens must be assessed for viability during specimen processing, prior to staining and fixation, on a non- fixed aliquot of the specimen’s single cell suspension; <ul style="list-style-type: none"> i. specimens that are less than fifty (50) percent viability must be rejected, and a replacement specimen shall be requested; and c) non-malignant leukocyte immunophenotyping specimens that have exceeded the laboratory established allowable holding time or were shipped during conditions of extreme heat or cold; <ul style="list-style-type: none"> i. specimens that are less than fifty (50) percent viability must be rejected, and a replacement specimen requested. 	<p>In the event that a specimen is irreplaceable or cannot be re-drawn, criteria must be included in the laboratory standard operating procedure to delineate how the patient specimen should be handled.</p> <p>If the blood specimen for non-malignant leukocyte immunophenotyping is collected into a tube containing a preservative (e.g., Streck Cyto-Chex BCT), viability is not required.</p> <p>In some cases (e.g., CSF), an extremely low cell count may not allow viability to be analyzed.</p>
<p>Cellular Immunology Standard of Practice 5 (CI S5): Viability Reporting</p> <p>In addition to the requirements in Reporting Standard of Practice 2, when viability testing is performed, the laboratory must include on the report:</p>	

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>a) the viability percentage; and</p> <p>b) for specimens less than eighty (80) percent viable, a statement that the results are based on a sample that was partially compromised due to the presence of greater than twenty (20) percent non-viable leukocytes.</p>	
<p>Cellular Immunology Standard of Practice 6 (CI S6): Reference Range Requirements</p> <p>For leukocyte function and non-malignant leukocyte immunophenotyping, the laboratory must follow manufacturer instructions for FDA approved, cleared or exempt instrument or test system operation and control according to Test Performance Specifications Standard of Practice 1.</p> <p>For any laboratory developed test (LDT), in addition to the requirements in Test Performance Specifications Standard of Practice 2, the laboratory must determine laboratory-derived reference ranges using a minimum of twenty-five (25) known healthy donors. The donor demographic records must include age and sex.</p>	<p>Reference ranges should be compared to published ranges to verify the expected values.</p> <p>Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p>
<p>Cellular Immunology Standard of Practice 7 (CI S7): Required Performance Checks of the Flow Cytometer</p> <p>The laboratory must follow manufacturer instructions for FDA approved, cleared or exempt instrument or test system operation and control.</p> <p>For laboratory developed tests (LDTs), on each day of use, and after maintenance procedures and repairs, acceptable instrument performance using fluorochrome-labeled beads</p>	<p>The manufacturer’s recommended procedures should be strictly followed for all FDA approved flow cytometers.</p> <p>Information on Departmental approval of a laboratory developed test (LDT) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p>

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>must be confirmed and documented to include:</p> <ul style="list-style-type: none"> a) compensation values for spectral overlap for each fluorochrome that is used for testing, utilizing beads labeled with fluorochrome-conjugated antibodies; and b) 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. 	
<p>Cellular Immunology Standard of Practice 8 (CI S8): Antibody Lot Assessments</p> <p>The laboratory must test each new lot of antibody or ligand to ensure that the mean fluorescent intensity (MFI) values for each population analyzed to ensure:</p> <ul style="list-style-type: none"> a) that manufacturer requirements are met for FDA approved, cleared or exempt tests; or b) acceptability criteria are met for laboratory developed tests (LDT). 	<p>Information on Departmental approval of a laboratory developed test (LDT) is available at:</p> <p>https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p>
<p>Cellular Immunology Standard of Practice 9 (CI S9): Antibody Fluorochrome Stability</p> <p>In addition to the requirements in Test Procedure Content Standard of Practice 1, the laboratory must have a procedure for:</p> <ul style="list-style-type: none"> a) protection of staining reagents from light, including room lighting conditions during the staining process; 	<p>Fluorochromes are sensitive to photobleaching (room lighting conditions) and/or undergo emission spectra changes by prolong exposure to fixatives like paraformaldehyde (formaldehyde).</p>

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<ul style="list-style-type: none"> b) storage of the stained tube until data acquisition on the flow cytometer; c) when reagents with different stabilities are combined, the shortest stability length shall be used for the combined reagents; and d) dependent on the fluorochrome-conjugated antibody combinations or other additives, follow manufacturer requirements on time restrictions between staining cells and flow cytometric analyses. 	
<p>Cellular Immunology Standard of Practice 10 (CI S10): Specimen Quality Assurance</p> <p>To ensure testing accuracy prior to reporting, the laboratory must document that:</p> <ul style="list-style-type: none"> a) each antibody gives consistent results in replicates; and b) any normal leukocyte population within the specimen is analyzed concurrently to ensure normal staining activities. 	<ul style="list-style-type: none"> 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.
<p>Cellular Immunology Standard of Practice 11 (CI S11): Compensation Calculation</p> <p>In the absence of manufacturer instructions, the laboratory must:</p> <ul style="list-style-type: none"> a) calculate general electronic compensation using beads labeled with fluorochrome-conjugated antibodies, and the features provided in the flow cytometer software to 	<p>General electronic compensation values obtained using beads may not be appropriate for all antibody combinations, and compensation may need to be recalculated using cells.</p> <p>The accurate determination of cellular antigen expression assists the identification of aberrant populations, underscoring the importance of accurate compensation calculation.</p>

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>calculate automatic compensation;</p> <p>b) confirm the accuracy of compensation values using a subset of labeled cells, when applicable;</p> <p>c) update compensation using labeled cells at a frequency determined by the director or individual delegated in writing by the director; and</p> <p>d) use antibody cocktail specific compensation when:</p> <ol style="list-style-type: none"> i. different fluorochromes are used in the same channel, e.g., FITC and Alexa Fluor 488; and ii. values (settings) of PMT voltages are specific for PNH analysis of RBC vs. WBC. 	
<p>Cellular Immunology Standard of Practice 12 (CI S12): Data Acquisition – Population Resolution and Gating</p> <p>For any laboratory developed test (LDT), the laboratory must:</p> <ol style="list-style-type: none"> 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. 	<p>Information on Departmental approval of a laboratory developed test (LDT) is available at:</p> <p>https://www.wadsworth.org/regulatory/clip/clinical-labs/obtain-permit/test-approval.</p> <p>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.</p>

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
<p>Cellular Immunology Standard of Practice 13 (CI S13): Event Collection Procedure</p> <p>In addition to the requirements in Test Procedure Content Standard of Practice 1, the laboratory must have standard operating procedures describing event collection when performing non-malignant and malignant leukocyte immunophenotyping.</p> <p>The procedure(s) must ensure that a statistically significant number of events are collected to provide accurate and reliable results.</p> <p>For rare event analysis, the lower limit of enumeration must be validated.</p>	<p>Non-malignant leukocyte immunophenotyping:</p> <ul style="list-style-type: none"> • for single-platform methods that use bead counts, bead event collection should be 1000 or greater per sample tube; • with the exception of CD4, at least 10,000 lymphocytes should be collected per sample tube for quantification; • when performing CD34 stem cell analysis, the laboratory should collect at least 100 stem cell events per sample for quantification; • for classical PNH analysis, a minimum of 10,000 events should be collected for each population analyzed; and • for leukocyte Adhesion Deficiency (unstimulated expression), a minimum of 5,000 events should be collected per population analyzed. <p>Malignant leukocyte immunophenotyping:</p> <ul style="list-style-type: none"> • 20,000 leukocyte events or 10,000 if the specimen presents as a single population, excluding cellular debris and dead cells; • high sensitivity analysis for glycosylphosphatidylinositol (GPI) anchorage of Paroxysmal Nocturnal Hemoglobinuria (PNH) should be used for the detection of specimens containing less than 1 to 0.01 percent of events: <ul style="list-style-type: none"> ○ with a minimum of 250,000 events for each population analyzed; ○ two (2) parameter density plots of both GPI markers

Cellular Immunology	
Cellular Immunology Categories	
Standard	Guidance
	to determine the double negative events; and <ul style="list-style-type: none">• 500,000 leukocyte events per tube for Minimal Residual Disease (MRD) analysis.

Leukocyte Function

Cellular Immunology	
Leukocyte Function	
Standard	Guidance
<p>Cellular Immunology – Leukocyte Function Standard of Practice 1 (LF S1): Limit of Endotoxin Levels</p> <p>Laboratories must procure media, reagents and glassware that contains < 0.5 endotoxin units (EU/mL).</p>	
<p>Cellular Immunology – Leukocyte Function Standard of Practice 2 (LF S2): Serum Component of Media</p> <p>If human serum is used for a functional assay, autologous or AB serum must be used.</p>	<p>Red cells and red cell membranes are common contaminants of extracted cellular material. Use of other blood type plasma could cause agglutination, if mismatched.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 3 (LF S3): Reagent Verification</p> <p>In addition to the requirements in Reagents and Media Standard of Practice 2, lot-to-lot evaluation of all new lots of reagents must use a normal control specimen to ensure reagents give results within laboratory-derived reference range values.</p>	<p>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.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 4 (LF S4): Biological Safety Cabinet Requirement</p> <p>The laboratory must use aseptic techniques during all steps of cell culture set-up and manipulation using a biological safety cabinet (BSC).</p>	<p>Refer to Laboratory Safety Standard of Practice 11 for additional requirements on the use of a BSC.</p>

Cellular Immunology	
Leukocyte Function	
Standard	Guidance
<p>Cellular Immunology – Leukocyte Function Standard of Practice 5 (LF S5): Normal Control Requirements</p> <p>A whole blood specimen or a fraction thereof (e.g., peripheral blood mononuclear cells (PBMC) for function) that is expected to fall within the laboratory’s established reference range for normal specimens must be included:</p> <ul style="list-style-type: none"> 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 for a laboratory developed test (LDT); and d) be stored under conditions as similar as possible to those of the test specimens. 	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at:</p> <p>https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p> <p>c) Validation studies for laboratory developed tests (LDTs) for each anticoagulant must receive approval by the Department.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 6 (LF S6): Function Quality Control – Negative, Positive, and Multi-level Controls</p> <p>In addition to the requirements in Test Procedure Content Standard of Practice 1, the laboratory must include in each analytic run or plate:</p> <ul style="list-style-type: none"> a) negative and positive controls for each functional assay; and 	<p>Positive controls demonstrating multiple levels of function must also be used when available.</p>

Cellular Immunology	
Leukocyte Function	
Standard	Guidance
<p>b) media, diluent, or carrier solutions cultured with each specimen without the assay stimulant.</p>	
<p>Cellular Immunology – Leukocyte Function Standard of Practice 7 (LF S7): Effector to Target Cellular Ratios</p> <p>The laboratory must include three (3) different ratios of effectors to targets determined to be in the optimal range.</p>	<p>Assays using effector to target ratios include Alloantigen, Cytolytic, and Phagocytosis analyses.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 8 (LF S8): Specimen Precision for Functional Analysis</p> <p>The laboratory must:</p> <ul style="list-style-type: none"> a) test specimens in duplicate for functional assays; b) test specimens in triplicate for proliferation and cytolytic assays if sufficient specimen is available; and c) reject the results and request a new specimen if the coefficient of variation among results for a similarly prepared specimen exceeds twenty (20) percent. 	
<p>Cellular Immunology – Leukocyte Function Standard of Practice 9 (LF S9): Stimulant Concentrations</p> <p>For each specimen in each run of functional assays the laboratory must test at least two (2) stimulant concentrations in the optimal response range, if there are sufficient cells.</p>	

Cellular Immunology	
Leukocyte Function	
Standard	Guidance
<p>Cellular Immunology – Leukocyte Function Standard of Practice 10 (LF S10): Function Assessment</p> <p>Each patient specimen must be set up for each analytical run to include stimulated and unstimulated conditions.</p>	<p>The optimal storage condition and life span of functional stimulants should be determined during the validation studies.</p> <p>Cells being assessed for functional activity should be characterized and the immunophenotype reported whenever possible.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 11 (LF S11): Evaluation during Functional Peak</p> <p>For <i>in vitro</i> functional assays, the laboratory must examine and test each functional activity during the validated peak activity interval.</p>	
<p>Cellular Immunology – Leukocyte Function Standard of Practice 12 (LF S12): Verification of Target Cell Labeling</p> <p>The laboratory must:</p> <ul style="list-style-type: none"> a) define acceptable spontaneous label release; and b) monitor and document for each run. 	<p>Assays using target labeling include Cytolytic and Phagocytosis.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 13 (LF S13): Proliferation Reporting</p> <p>In addition to the requirements in Reporting Standard of Practice 2, for proliferation assays, the laboratory must report results as responsive or non-responsive.</p> <p>When quantifying proliferation in response to a mitogen or antigen, an assay will be considered responsive if the</p>	<p>For mitogen induced proliferation, the normal response to mitogen is expected to induce a positive stimulation response.</p> <p>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</p>

Cellular Immunology	
Leukocyte Function	
Standard	Guidance
<p>results are two (2) standard deviations (SD) above the negative control result.</p>	<p>not within one or two standard deviations of the mean value for the healthy unexposed control population.</p> <p>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.</p> <p>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.</p>
<p>Cellular Immunology – Leukocyte Function Standard of Practice 14 (LF S14): Reporting Flow Cytometric Results for Functional Analysis</p> <p>In addition to the requirements in Reporting Standard of Practice 2, laboratories using flow cytometric analysis of function must report:</p> <ul style="list-style-type: none"> a) the characterized population analyzed; and b) changes in biomarker expression due to stimulation including percentage and the quantitative change in mean fluorescence intensity (MFI). 	

Non-Malignant Leukocyte Immunophenotyping

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 1 (NM S1): Single Platform Requirements</p> <p>In addition to the requirements in Test Procedure Content Standard of Practice 1, when the laboratory uses single-platform methods that use fluorescent bead counts, the laboratory must have a procedure that requires the use of a lyse/no wash procedure.</p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 2 (NM S2): Positive Control Requirements</p> <p>For each non-malignant leukocyte immunophenotyping marker being assayed, control materials suitable for error detection throughout the reportable range must be used on each day of testing to include:</p> <ul style="list-style-type: none"> a) two (2) levels of commercial controls when available at two (2) different concentrations within the reportable range; or b) one (1) commercial and one (1) whole blood specimen from a donor, if consistent with manufacturer instructions, when the commercial control levels do not meet the requirements above; or c) a freshly prepared whole blood specimen in a validated anticoagulant from a donor when commercial controls 	<p>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.</p> <p>If sample preparation problems occur, additional control specimens should be used for trouble-shooting purposes.</p> <p>The whole blood normal control for each assay must be stored under conditions as similar as possible to those of the test specimens.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
are not available, if allowed by the manufacturer.	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 3 (NM S3): Positive-Negative Bio-marker Determination</p> <p>For laboratory developed tests (LDTs), the laboratory must:</p> <ul style="list-style-type: none"> a) use negatively stained cells within the same gated population, when using SS/CD45 gating, to determine 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) must be isotype matched antibody at similar concentrations and fluorochrome to protein (F/P) ratios as the test antibody. 	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p> <ul style="list-style-type: none"> 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. <p>The isotype control is the negative control to detect non-specific antibody binding.</p>
Lymphocyte Enumeration (including T-Lymphoid Analysis)	
<i>All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed.</i>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 4 (NM S4): Lymphocyte enumeration – Quality Control using CD3 Tube Replicate</p> <p>When using multitube panels, with a common biomarker such as CD3, the percentage values must not differ by more than</p>	<p>The use of different fluorochromes or different monoclonal antibodies for CD3 could affect this determination.</p> <p>When CD3 values do not replicate, the laboratory should document that the specimen was repeated and/or re-stained.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
three (3) and absolute values must not differ by greater than ten (10) percent.	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 twenty (20) percent.
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 5 (NM S5): Lymphocyte enumeration – Quality Control using Lymphosum Determination</p> <p>When a lab analyzes T, B and NK cells, the lymphosum must be within 90 -105 percent.</p>	<p>Lymphosum refers to the sum of all subsets of lymphocytes (CD3⁺ plus CD19⁺ plus CD3⁻/CD56⁺ and/or CD16⁺ cells).</p> <p>The laboratory should troubleshoot for technical difficulties when the lymphosum is out of optimal range.</p>
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 6 (NM S6): Lymphocyte enumeration – Quality Control using T-Sum Determination</p> <p>The T-sum for laboratory developed tests (LDTs) must be monitored as follows:</p> <p>a) 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⁻) must not exceed a difference of 3 of the total CD3 percentage mean.</p>	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p> <p>If a greater difference is found, the laboratory should repeat the analysis, including re-staining, to confirm that no preparation problems occurred.</p> <p>Double positive (DP) CD4⁺CD8⁺ T lymphocytes may impact T-sum determination if this DP T cell subset has not been resolved.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 7 (NM S7): Lymphocyte Enumeration – Reporting Requirements</p> <p>In addition to the requirements in Reporting Standard of Practice 2, unless the single platform instrumentation only provides absolute numbers, percentages and absolute numbers of lymphocyte subsets must be reported.</p>	
CD34 Stem Cell Enumeration	
<i>All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed.</i>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 8 (NM S8): CD34 Stem Cell Enumeration – Specimen Age</p> <p>For CD34 stem cell enumeration, laboratories must process specimens within the following age limits:</p> <ul style="list-style-type: none"> a) for FDA approved assays, the manufacturer’s recommendations for maximum specimen age cut-offs for the specimen type and the assay testing system used; or b) for any laboratory developed test (LDT), the laboratory must have a procedure for testing specimens for CD34 stem cell enumeration within the time frame validated and approved by the Department. 	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at:</p> <p>https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 9 (NM S9): CD34 Stem Cell Enumeration – Apheresis Specimen Requirements</p> <p>The cell number of apheresis specimens must be determined prior to staining. If dilution is necessary, the laboratory must include a support protein in the dilution buffer. The dilution factor must be documented and used for total CD34 cell count calculations.</p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 10 (NM S10): CD34 Stem Cell Enumeration – Specimen Staining/Processing Reagent Requirements</p> <p>Unless otherwise instructed by the manufacturer for FDA approved or cleared tests, the laboratory must:</p> <ul style="list-style-type: none"> a) use CD34 antibody reagent that: <ul style="list-style-type: none"> i. binds CD34 class II or class III epitopes; ii. be conjugated with a bright fluorochrome (e.g., PE); and b) use CD45 antibody reagent that detects all isoforms and glycoforms; and c) use an erythrocyte lyse reagent without fixative in a lyse/no wash manner. 	

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 11 (NM S11): CD34 Stem Cell Enumeration – Viable Cell Assessment</p> <p>CD34 stem cell enumeration must include the simultaneous determination of viable cells.</p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 12 (NM S12): CD34 Stem Cell Enumeration – Control Requirements</p> <p>For the enumeration of CD34 stem cells, low and high-level controls must be assessed on each day of testing.</p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 13 (NM S13): CD34 Stem Cell Enumeration – Reporting Requirements</p> <p>In addition to the requirements in Reporting Standard of Practice 2, viable CD34 stem cells must be quantified and reported as absolute number of viable cells per microliter.</p>	

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Analysis of GPI anchored proteins for PNH diagnosis <i>All the general standards for Non-Malignant Leukocyte Immunophenotyping must also be followed.</i></p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 14 (NM S14): Paroxysmal Nocturnal Hemoglobinuria Diagnosis – Specimen Stability</p> <p>Specimens for glycosylphosphatidylinositol (GPI) anchorage of Paroxysmal Nocturnal Hemoglobinuria (PNH) immunophenotyping must be processed within twenty-four (24) hours of collection, if kept at room temperature, or forty-eight (48) hours if kept at 2-8 degrees Celsius.</p>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 15 (NM S15): Paroxysmal Nocturnal Hemoglobinuria Diagnosis – Analysis Requirements</p> <p>A control blood specimen must be stained and analyzed concurrently with patient specimen to define normal expression in the analysis of glycosylphosphatidylinositol (GPI) anchored antigens.</p>	<p>Both specimens (normal and patient) should be collected within four (4) hours of each other since antigens are naturally shed post-collection.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 16 (NM S16): Paroxysmal Nocturnal Hemoglobinuria Diagnosis – Positive Staining Control</p> <p>Analysis for Paroxysmal Nocturnal Hemoglobinuria (PNH) diagnosis must 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) must be documented.</p>	<p>Use of a lineage marker for this purpose can also provide assistance for population identification and gating (e.g., for WBC’s CD45, neutrophils CD15, and/or monocytes CD64; for RBC’s- anti-glycophorin A CD235a).</p>
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 17 (NM S17): Paroxysmal Nocturnal Hemoglobinuria Diagnosis – Review Criteria and Reporting</p> <p>In addition to the requirements in Reporting Standard of Practice 2, reports must include an interpretative statement related to the impact of any recent blood transfusions.</p> <p>The total PNH content per cell population must be reported while acknowledging complete (type III) or partial (type II) deficiency components.</p> <p>Disease diagnosis confirmation requires an expression deficiency of at least two (2) different GPI biomarkers including monoclonal antibodies and/or FLAER directed against two (2) different GPI-anchored antigens assessed on a minimum of two (2) different cell lineages (e.g., RBC and neutrophils).</p>	<p>Transfused donor cells will dilute the patient’s blood phenotype composition.</p> <p>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.</p>

Cellular Immunology	
Non-Malignant Leukocyte Immunophenotyping	
Standard	Guidance
Leukocyte Adhesion Deficiency (unstimulated)	
<i>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.</i>	
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 18 (NM S18): Leukocyte Adhesion Deficiency (unstimulated expression) – Positive Staining Control</p> <p>Analysis for Leukocyte Adhesion Deficiency (LAD) must 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) must be documented.</p>	<p>These markers should be used for leukocyte population identification for gating.</p> <p>Analysis for LAD Type 1 should include markers for the leukocyte β2 integrins (staining panels of CD18 with CD11a and CD11b.</p> <p>Analysis for LAD Type 2 should include Sialyl Lewis x, (CD15s).</p>
<p>Cellular Immunology – Non-Malignant Leukocyte Immunophenotyping Standard of Practice 19 (NM S19): Leukocyte Adhesion Deficiency (unstimulated expression) – Report Requirements</p> <p>In addition to the requirements in Reporting Standard of Practice 2, leukocyte populations must be identified and the biomarkers used to characterize them. Reports must include the percentage of cells positive for each biomarker.</p>	

Malignant Leukocyte Immunophenotyping

Cellular Immunology	
Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Malignant Leukocyte Immunophenotyping Standard of Practice 1 (ML S1): Leukocyte Count Adjustment</p> <p>The laboratory must quantify leukocyte concentrations and adjust as necessary to achieve optimal cell-to-reagent ratio and instrument acquisition event rate. The laboratory must document the initial and final leukocyte concentrations and any dilution calculations.</p>	
<p>Cellular Immunology – Malignant Leukocyte Immunophenotyping Standard of Practice 2 (ML S2): Periodic Verification</p> <p>A freshly prepared whole blood specimen from a healthy donor must be tested as a normal control at least monthly for malignant leukocyte immunophenotyping and results must be documented.</p> <p>The normal control must be used to evaluate:</p> <ul style="list-style-type: none"> 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. 	<p>Cell lines should also be used for the assessment of antibody reagents that are not positive on normal leukocyte populations.</p>

Cellular Immunology	
Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>Cellular Immunology – Malignant Leukocyte Immunophenotyping Standard of Practice 3 (ML S3): Positive-Negative Bio-marker Determination</p> <p>To assess biomarker expression as positive or negative the laboratory must use:</p> <ul style="list-style-type: none"> a) negative staining cells within the same gated population if the antibodies are isotype-matched; b) non-stained cells as controls when fluorescence minus one (FMO) antibody combinations are used; and c) when analyzing cellular antigens of dim fluorescent intensity, isotype control antibodies must be used to set cursors that distinguish negative from positive staining cells. 	<p>These negative controls must be isotype-matched at similar concentrations and fluorochrome to protein (F/P) ratios as the test antibody.</p>
<p>Cellular Immunology – Malignant Leukocyte Immunophenotyping Standard of Practice 4 (ML S4): Report Requirements</p> <p>In addition to the requirements in Reporting Standard of Practice 2, the leukemia/lymphoma report must include:</p> <ul style="list-style-type: none"> 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 	<p>The flow cytometric results should be correlated with pathology results, when available, and whenever appropriate, should indicate the monoclonality and/or bi-phenotypic characteristics.</p> <p>The analysis of the aberrant population should be extensive enough as to allow for future detection of minimal residual disease.</p>

Cellular Immunology	
Malignant Leukocyte Immunophenotyping	
Standard	Guidance
<p>or lower) than on normal cells of similar hematopoietic lineage. The patient report must include a description of the quality of staining (e.g., dim or low intensity, bright or high intensity);</p> <p>e) description of lineage and stage for each identified aberrant population; and</p> <p>f) declaration of any specimen condition that was less than optimal.</p>	
<p>Cellular Immunology – Malignant Leukocyte Immunophenotyping Standard of Practice 5 (ML S5): Minimal Residual Disease Analysis – Antibody Panel Design and Analysis Requirements</p> <p>Requirements for Antibody Panel Design and Analysis for Minimal Residual Disease Analysis must include:</p> <p>a) antibody selection and gating strategy based on patient’s relevant medical history, including any previous immunophenotypic analysis;</p> <p>b) antibody panels capable of detecting immunophenotypic shifts comprised of a minimum of four (4) colors and redundant fluorescent antibodies between the stained tubes;</p> <p>c) sequential gating to detect the rare event aberrant cell and discriminate normal mature cells and normal regenerating progenitor cells;</p> <p>d) documentation of specimen collection timing.</p>	<p>b) Six (6) 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 fluorescein isothiocyanate (FITC) and phycoerythrin (PE) for the most critical aberrancy biomarkers with PE being used for the low expression biomarkers.</p>

Cytokines

Cytokines	
Standard	Guidance
<p>Cytokine Standard of Practice 1 (CK S1): Reference Range</p> <p>Laboratories must establish or verify the reference range for each cytokine for each matrix tested.</p>	<p>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.</p>
<p>Cytokine Standard of Practice 2 (CK S2): Linear Range</p> <p>All results that fall above the reportable range for the method (highest point on the linear portion of standard curve) must be diluted and retested.</p>	
<p>Cytokine Standard of Practice 3 (CK S3): Duplicate Analysis</p> <p>The laboratory must test all specimens in duplicate with non-automated methods unless a laboratory developed test (LDT) demonstrates acceptable precision and has received approval from the Department.</p>	<p>Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.</p> <p>Laboratories should establish an acceptable range of variation for duplicate values (e.g., less than twenty (20) percent coefficient of variation).</p>