Proposed Cellular Immunology and Cytokines Standards – Comments and Responses

Proposed Standards were made available to New York State permitted laboratories and laboratories in application for a permit on March 4th, 2020. The announcement was by e-mail to the facility and laboratory contact person's e-mail address and the Proposed Standards were posted to the CLEP website.

The comment period ended June 15th, 2020. Comments received from any regulated parties and responses are shown here.

Standards will be adopted July 13th, 2020, with an effective date of August 1st, 2020.

General Cellular Immunology and Cytokines Standards Comments

COMMENT 1:
Does the deletion of CILF S24 mean results reporting reverts to the General System Standards?

RESPONSE 2:
All General Systems Standards, including reporting requirements, apply to Cellular Immunology Category testing, if applicable. There is no change to the standards based on the comment received.

COMMENT 2:
For Cellular Immunology and Cytokines general requirements, please confirm that the requirements for "30 hours if using EDTA anticoagulant and 48 hours if using ACD or heparin anticoagulant" in CINM S2, CINM S3 and CINM S4 have been removed in the proposed CI standards. Do the proposed CI standards now indicate only that "specimens must be assessed for viability" with indications for rejection based on the viability?

RESPONSE 2:
The standards were revised and now indicate that specimens must be assessed for viability. There is no change to the standards based on the comment received.

Cellular Immunology and Cytokines Standards Comments

Cellular Immunology

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 2 (CI S2): Client Instructions for Specimen Transport and Storage Prior to Analysis In addition to the requirements in Specimen Processing Standard of Practice 1, the laboratory must provide specimen transport instruction to clients, in the absence of manufacturer instructions, indicating requirements for: a) specimen transport temperatures of 18-25 degrees Celsius for all specimens, except for: 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.	Information on Departmental approval of laboratory developed tests (LDTs) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.

Cellular Immunology Standard of Practice 2 (CI S2): Client Instructions for Specimen Transport and Storage Prior to Analysis

COMMENT:

Please provide guidance for how to calculate allowable transport time.

RESPONSE:

For assays not specified in the standard, the laboratory must (1) follow manufacturer instruction when provided or (2) for an LDT, the director or person delegated in writing by the director must determine the transport time based on empirical evidence. There is no change to the standard based on the comment received.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 4 (CI S4): Specimen Viability Testing The lab must perform viability testing prior to testing on all: 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; 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	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. 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. In some cases (e.g., CSF), an extremely low cell count may not allow viability to be analyzed.

specimens that have exceeded the laboratory established allowable holding time or were shipped during conditions of extreme heat or cold; i. specimens that are less than fifty (50) percent viability must be rejected, and a replacement specimen requested.	

Cellular Immunology Standard of Practice 4 (CI S4): Specimen Viability Testing

COMMENT 1:

- Cellular Immunology Standard of Practice 4 (CI S4): Specimen Viability Testing, Page 6 This new requirement now includes stem cells. Please clarify what is meant by performing viability testing before testing. The actual testing our laboratory performs is to determine stem cell enumeration and viability. This new standard is not feasible for the assay we perform.

RESPONSE 1:

The laboratory must comply with all applicable New York State Clinical Laboratory Standards of Practice. There is no change to the standard based on the comment received.

COMMENT 2:

Is lab established allowable holding time the same as validated specimen stability?

RESPONSE 2:

Holding time is a component of specimen stability. Other factors affecting stability may include, for example, collection tubes, additives, anticoagulants, transport and storage temperatures, etc. There is no change to the standard based on the comment received.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 5 (CI S5): Viability Reporting In addition to the requirements in Reporting Standard of Practice 2, when viability testing is performed, the laboratory must include on the report: a) the viability percentage; and 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.	

Cellular Immunology Standard of Practice 5 (CI S5): Viability Reporting

COMMENT:

- Cellular Immunology Standard of Practice 5 (CI S5): Viability Reporting, Page 8
  The point of our laboratory's test is to verify viability of the product. The product tested is not compromised. Please clarify if the intent is to reject the sample.

RESPONSE:

The intent is to include required information related to specimen viability on the report. There is no change to the standard based on the comment received.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 7 (CI S7): Required Performance Checks of the Flow Cytometer The laboratory must follow manufacturer instructions for FDA approved, cleared or exempt instrument or test system operation and control. For laboratory developed tests (LDTs), on each day of use, and after maintenance procedures and repairs, acceptable instrument performance using fluorochrome-labeled beads must be confirmed and documented to include: 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.	The manufacturer's recommended procedures should be strictly followed for all FDA approved flow cytometers. 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.

Cellular Immunology Standard of Practice 7 (CI S7): Required Performance Checks of the Flow Cytometer

COMMENT:

Is compensation expected to be performed every day testing is run for all cellular, non-malignant phenotyping, and malignant phenotyping LDTs?

RESPONSE:
For LDTS, compensation must be performed on each day of testing. There is no change to the standard based on the comment received.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 8 (CI S8): Antibody Lot Assessments 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: a) that manufacturer requirements are met for FDA approved, cleared or exempt tests; or b) acceptability criteria are met for laboratory developed tests (LDT). ~~do not differ more than fifteen (15) percent.~~	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.

Cellular Immunology Standard of Practice 8 (CI S8): Antibody Lot Assessments

COMMENT:
Does this apply to FDA approved reagents/test kits?

RESPONSE:
Based on the comment received the standard has been revised to indicate requirements for FDA approved cleared or exempt tests and LDTs.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 11 (CI S11): Compensation Calculation In the absence of manufacturer instructions, the laboratory must: a) calculate general electronic compensation using beads labeled with fluorochrome-conjugated antibodies, and the features provided in the flow cytometer software to calculate automatic compensation; b) confirm the accuracy of compensation values using a subset of labeled cells, when applicable; c) update compensation using labeled cells at a frequency determined by the director or individual delegated in writing by the director; and d) use antibody cocktail specific compensation when: 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.	General electronic compensation values obtained using beads may not be appropriate for all antibody combinations, and compensation may need to be recalculated using cells. The accurate determination of cellular antigen expression assists the identification of aberrant populations, underscoring the importance of accurate compensation calculation.

Cellular Immunology Standard of Practice 11 (CI S11): Compensation Calculation

COMMENT:

We ask that instead of "director" it state:
laboratory director, or assistant director(s) holding an appropriate certificate of qualification.

RESPONSE:

This responsibility may be delegated by the director. The laboratory director is responsible for ensuring that delegated responsibilities are performed by staff (CLIA 493.1407(b) and 10NYCRR 19.3(c)). The standard has been revised based on the comment received.

Cellular Immunology	
Cellular Immunology Categories	
Proposed Standard	***Proposed Guidance***
Cellular Immunology Standard of Practice 13 (CI S13): Event Collection Procedure 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. The procedure(s) must ensure that a statistically significant number of events are collected to provide accurate and reliable results. For rare event analysis, the lower limit of enumeration must be validated.	Non-malignant leukocyte immunophenotyping: • 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. Malignant leukocyte immunophenotyping: • 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 glycosylphosphatidylinosistol (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: ○ with a minimum of 250,000 events for each population analyzed; ○ two (2) parameter density plots of both GPI markers to determine the double negative events; and • 500,000 leukocyte events per tube for Minimal Residual Disease (MRD) analysis.

Cellular Immunology Standard of Practice 13 (CI S13): Event Collection Procedure

COMMENT:

1. What about non-malignant immunophenotyping assays where labs may be testing patient populations or sample types for which they cannot collect 10,000 lymphocytes (ex. Transplant monitoring, BAL) or where the FDA approved software can't be changed to count 10,000 lymphs?
2. Does the exception for CD4 testing extend to all lymphocyte subset testing (ex. TBNK) performed using FDA approved software and reagents?

RESPONSE:

Laboratories must follow manufacturer instructions. In the absence of manufacturer instruction, the guidance provides recommended practices. There is no change to the standard based on the comment received.

Cytokines

Cytokines	
Proposed Standard	***Proposed Guidance***
Cytokine Standard of Practice 2 (CK S2): Linear Range 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.	~~False positive results may be obtained when the specimen is run neat due to matrix interference.~~

Cytokine Standard of Practice 2 (CK S2): Linear Range

COMMENT:

Please clarify:

- Why would a matrix effect cause an incorrect result if the patient result is > AMR? Are there literature data or instances where this has occurred?
- Suggest removing the word "neat" (research term) and replace with "undiluted".

RESPONSE:

The guidance has been deleted based on the comment received.