CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Method and Panel Components Change for Flow Cytometry Immunophenotyping for Leukemia and Lymphoma (FCI)

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Testing Schedule: Monday through Friday morning and afternoon, Saturday morning

Clinical use:
CPAL will be switching flow cytometry platforms (and associated panels) from the current 6-color BD Canto II to the new 10-color Beckman Coulter Navios. Flow cytometry immunophenotyping (FCI) is a testing method that allows multi-parametric analysis of cell-surface and cytoplasmic antigens. FCI utilizes prepared single-cell suspensions, monoclonal antibodies conjugated to fluorescent dyes, and sophisticated instrumentation (flow cytometer) that provides a laser light source, a focused fluidics environment to carry the cell suspension through the light source, and an electronic signal detection and amplification system. As a result, FCI testing offers highly efficient and rapid evaluation of a submitted sample for a suspected hematologic malignancy.

The advantages of using FCI include 1) the ability to distinguish benign and neoplastic conditions, 2) the diagnosis and characterization of leukemias and lymphomas, 3) the capability to asses other neoplastic/pre-neoplastic disease states such as myelodysplastic syndrome and plasma cell dyscrasias, 4) the detection of residual disease in patients who have undergone a treatment regimen for a hematologic malignancy, and 5) provision of prognostic information for particular neoplastic states (i.e. CD38 expression in CLL patients).

Specimen: Requirements vary depending on specimen type.

- Peripheral Blood and Bone Marrow
  - Acceptable anticoagulants: EDTA, Sodium Heparin
  - Temperature requirements
    - Store and ship at ambient temperature
  - Specimen age
    - Receipt at CPAL within 24 hours of collection is requested
    - Specimens received 24 – 48 hours post-collection will be accepted
    - Specimens older than 48 hours post-collection will be rejected unless deemed irretrievable by attending physician (disclaimer will be attached to the generated data).
  - Volume requirements
    - Minimum acceptable volume = 1 mL
    - Every attempt will be made to process any amount of sample sent.
  - Other rejection criteria: evidence of freezing

- Surgical Biopsy
  - Acceptable suspension medium: RPMI
  - Temperature requirements
    - Store and ship at 2-8^{o}C
  - Specimen age
    - Receipt at CPAL within 24 hours of collection is requested
    - Specimens received within 24 – 48 hours post-collection will be accepted
    - Specimens older than 48 hours post-collection will be rejected unless deemed irretrievable by attending physician (disclaimer will be attached to the generated data).
  - Volume requirements
    - 1-5 tissue chunks or core material
    - Suspend tissue in 10x volume of RPMI
  - Other rejection criteria
    - evidence of freezing or fixation

- Fine Needle Aspirate (FNA) Biopsy
  - Acceptable suspension medium: RPMI
  - Temperature requirements
    - Store and ship at 2-8^{o}C
  - Specimen age
    - Receipt at CPAL within 24 hours of collection is requested
    - Specimens received within 24 – 48 hours post-collection will be accepted
    - Specimens older than 48 hours post-collection will be rejected unless deemed irretrievable by attending physician (disclaimer will be attached to the generated data).

- Volume requirements
  - Suspend tissue in 1-5 mL of RPMI
- Other rejection criteria
  - evidence of freezing or fixation

- Serous Effusions (Pleural, Peritoneal, Pericardial)
  - Temperature requirements
    - Store and ship at 2-8^{o}C
  - Specimen age
    - Receipt at CPAL within 24 hours of collection is requested
    - Specimens received within 24 – 48 hours post-collection will be accepted
    - Specimens older than 48 hours post-collection will be rejected unless deemed irretrievable by attending physician (disclaimer will be attached to the generated data).
  - Volume requirements
    - Minimum acceptable volume = 5 mL
  - Other rejection criteria
    - evidence of freezing or fixation

- CSF
  - Temperature requirements
    - Store and ship at 2-8^{o}C; do not freeze
  - Specimen age
    - Receipt at CPAL within 24 hours of being collected is **required**
    - Specimens older than 24 hours post-collection will be rejected unless deemed irretrievable by attending physician (disclaimer will be attached to the generated data).

- Paroxysmal nocturnal hemoglobinuria (PNH) is a now being performed using a High-Sensitivity assay for both WBCs and RBCs. Specimen requirement and strategy for testing is based on recommendations from the most current consensus documents for PNH testing. [6-7]

  - Peripheral blood collected in EDTA is the **only** acceptable specimen.
  - Bone marrow is not desirable because normal immature myeloid populations may express lower levels of GPI-anchored proteins, making interpretation difficult.
  - Specimens should be stored and shipped at ambient temperature
  - WBC analysis is best performed within 24-48 hours of collection due to degranulation of myeloid population.
  - RBC analysis may be performed up to 7 days post-collection if the sample is stored at 2-8^{o}C at the conclusion of WBC analysis.

Summary

- Sample submissions to CPAL for flow cytometry should include a copy of any collection or surgical forms, if available. All orders are placed through **LabNexus**.
- The strategy for testing is based on recommendations from the most current consensus documents for testing [1-5] as well as consultation from the Beckman Coulter representatives and members of the CPAL Flow Cytometry Affinity Group.
- The composition and extent of FCI testing is based on the ordering information transmitted through LabNexus. Reflexive testing will be performed based on results of initial studies, at either the discretion of CPAL flow cytometry staff, or at the instruction of the submitting pathologist after their review of results.

Table 1: Navios Flow Panel Matrix

NHL (inc CLL)		**Bkg**	**T1**	**B1**		
	CD5-/CD10- CD5+/CD23- if ALCL or g/d TCL suspect > 0.5% PC	B2 B2 T2 PCD				(reflexive)
CLL RD		**Bkg**	**B1**			
PCD		**Bkg**	**PCD**			
Blasts		**Bkg**	**MyeloLymph**	**AML**		
	ALL AML M6/M7	Cytoplasmic T1 M6/M7				(reflexive)
Cytopenia		**Bkg**	**T1**	**B1**	**MyeloLymph**	
High Sens. PNH (if WBC Pos, reflexed to RBC)		**Bkg**	**PNH-Gran**	**PNH-Mono**		
Mastocytosis		**Bkg**	**Mast**			
CamPath		**Bkg**	**Campath**			
LGL		**Bkg**	**T1**			

- FCI is performed using 10-color panels, utilizing monoclonal antibodies conjugated to fluorochromes (see Table 2 on following page). The panels are organized to contain a number of tubes, each consisting of 10 (some less) reagents combined in a manner that systematically examines antigens expressed on leukocyte subsets including:
  - Lymphocytes: T-, B-, NK-
  - Plasma cells
  - Monocytes
  - Granulocytes
  - Progenitor cells: lymphoid, myeloid

Table 2: Navios 10-Color Antibody Matrix

Tube	FITC	PE	ECD	PC5.5	PeCy7	APC	APC-AF700	APC-A750	PacBlue	KrO
Bkg	**	**	**	7AAD	**	**	**	**	**	CD45
T1	CD57	CD56	CD7	CD8	CD2	CD4	CD3	CD5	CD16	CD45
T2	CD4	CD30	**	CD8	TCR g/d	TCR a/b	CD3	CD25	**	CD45
B1	kappa	lambda	CD19	**	CD10	CD20	CD23	CD5	CD38	CD45
B2	FMC7	CD11c	CD19	CD22	CD10	CD103	**	CD5	CD25	CD45
PCD	(c)kappa	(c)lambda	CD19	CD138	CD56	CD117	**	**	CD38	CD45
MyeloLymph	HLA-DR	CD56	CD19	CD13	CD10	CD20	CD34	CD11b	CD16	CD45
AML	CD15	CD33	CD14	**	CD64	CD117	CD34	**	CD16	CD45
M6/M7	CD42a	CD71	**	**	CD61	CD36	CD34	CD235a	CD38	CD45
PNH-Mono	FLAER	CD14	**	CD64	CD45	**	**	**	**	**
PNH-Gran	FLAER	CD24	**	CD15	CD45	**	**	**	**	**
PNH-RBC	CD235a	CD59	**	**	**	**	**	**	**	**
Cytoplasmic	(n)TdT	©MPO	CD19	**	**	©CD79a	©CD3	**	**	CD45
Mast	HLA-DR	CD33	CD19	CD22	CD2	CD117	**	CD25	**	CD45
Campath	CD52	**	CD19	**	**	**	CD3	**	**	CD45

- Results are conveyed to the submitting institution through LabNexus:
  - PDF documents of flow cytometry histograms/plots, are placed on the LabNexus website (https://cpalmolpath.labnexus.net) for review by pathologists.
- Interpretation is generated by submitting pathologists.
  - Pathologist may order reflexive flow cytometry testing at CPAL, and/or reflexive ancillary testing at the reference lab after review of flow cytometry results.
- Patient charges are generated by the submitting hospital, not CPAL.
  - CPT codes to be used by the institution to generate patient charges include:
    - Technical component (Part A)
      - 88184 (Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker)
      - 88185 (Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker)
    - Professional component (Part B)
      - 88187 (Flow cytometry, 2-8 markers)
      - 88188 (Flow cytometry, 9-15 markers)
      - 88189 (Flow cytometry, 16 or more markers)

Validation Studies

The validation process for FCI testing began in June 2014 and was completed in October 2014.

For detailed analysis of the validation studies, or to discuss any aspect of FCI testing performed at CPAL, please call the laboratory contacts listed on first page or refer to www.cpallab.com/technotes for the validation document.

References

1) Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood* : 111 (8); 2008.

2) Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry B Clin Cytom.* 2007;72 Suppl 1:S14-22.

3) BioLegend website, www.biolegend.com "Expression of Common Surface Molecules on Blood Cells" and "Fluorophore Brightness Index."

4) BeckmanCoulter website, www.beckmancoulter.com , "Fluorophore Excitation and Emission Spectra" chart.

5) BD website, www.bdbiosciences.com , archived ppt presentation by Holden T. Maecker, "Design and Optimization of Multicolor Panels."

6) Sutherland DR, Keeney M, Illingworth A (2012) Practical guidelines for the high-sensitivity detection and monitoring of paroxysmal nocturnal hemoglobinuria clones by flow cytometry. *Cytometry B Clin Cytom*82(4): 195–208.

7) Raza A, Ravandi F, Rastogi A, Bubis J, Lim SH Weitz I, Castro-Malaspina H,Galili N, Jawde RA, Illingworth A. A Prospective Multicenter Study of Paroxysmal Nocturnal Hemoglobinuria Cells in Patients with Bone Marrow Failure. *Cytometry Part B* 2014:86B:175–182.