

Background on CAP Proficiency Testing for Leukemia/Lymphoma

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CAP PT

- Allows laboratories to regularly evaluate their performance and improve the accuracy of the patient results they provide.
- PT is required for the limited number of non-waived tests found in *Subpart I, Proficiency testing programs for nonwaived testing, of the CLIA regulations in 42CFR Part 493.*

CAP PT

- For non-regulated analytes (such as flow cytometry testing), CLIA requires laboratories to take steps to assure accuracy twice/yr. PT programs can serve meet this requirement.
- CAP, as an approved PT program, provides individual laboratories with unknown specimens for testing. The participants analyze the specimens and return the results to the CAP for evaluation.

CAP PT

- PT has value in
 - acting as an external quality measure
 - assisting in test method verification & staff continuing education
 - assisting the lab to improve when there are system/process/personnel problems with test performance
- After the testing event, PT samples have use as
 - Tool for competency assessment, training, education
 - Tool to assess quality and compare results at different sites in a health system

CAP Flow Cytometry PT Surveys (non-regulated analyte)

- FL-1
 - Basic lymphocyte subsetting
 - Immunodeficiency monitoring
- FL-2
 - DNA analysis
- FL-4
 - Hematopoietic progenitor cell counting

CAP Flow Cytometry PT Surveys Leukemia/Lymphoma (non-regulated analyte)

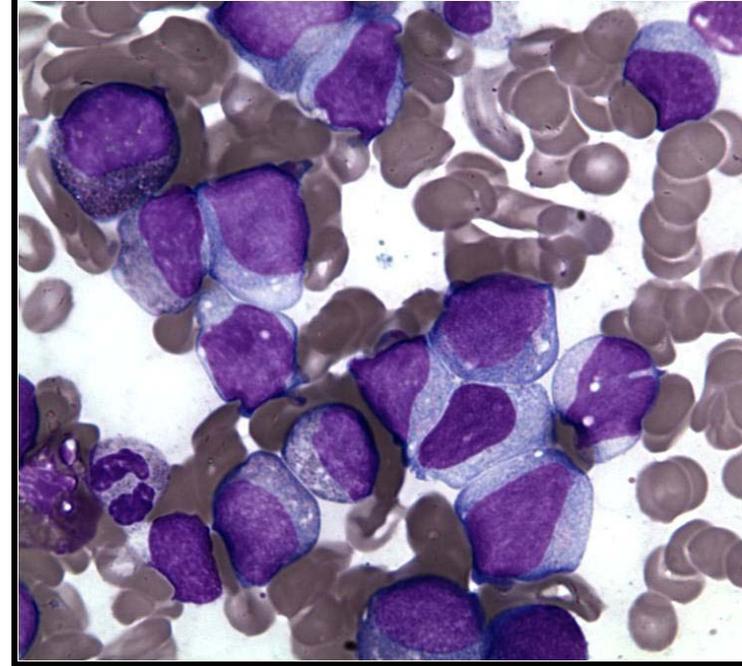
- FL3
 - Cell lines
- FL3CD
 - list mode data files
- FL5
 - interpretation only of gated dot plots

FL3 Survey

- Participants receive aliquot of a stabilized cell line as single cell suspension
- Data collected on preanalytic, analytic and post-analytic (interpretive) phases
- Approx. 525 laboratories

Instructions FL3

- Case Hx 2012 FL3B-03
- Instructions for participants
 - Given history and images provided, handle sample as you would a clinical sample
 - Report individual analyte results based on Bethesda consensus recommendation terminology
 - Report favored interpretation/diagnosis



Result form example

CD2	FL3-03	110 <input type="text"/>	120 <input type="text"/>	130 <input type="radio"/> 11 <input type="radio"/> 33	140 <input type="text"/>	150 <input type="text"/>
	FL3-04	160 <input type="text"/>	170 <input type="text"/>	180 <input type="radio"/> 11 <input type="radio"/> 33	190 <input type="text"/>	200 <input type="text"/>

Favored Interpretation

You must provide an interpretive answer. If you feel there was insufficient information available for assessment, please explain why in the space labeled "Other, specify."

FL3-03

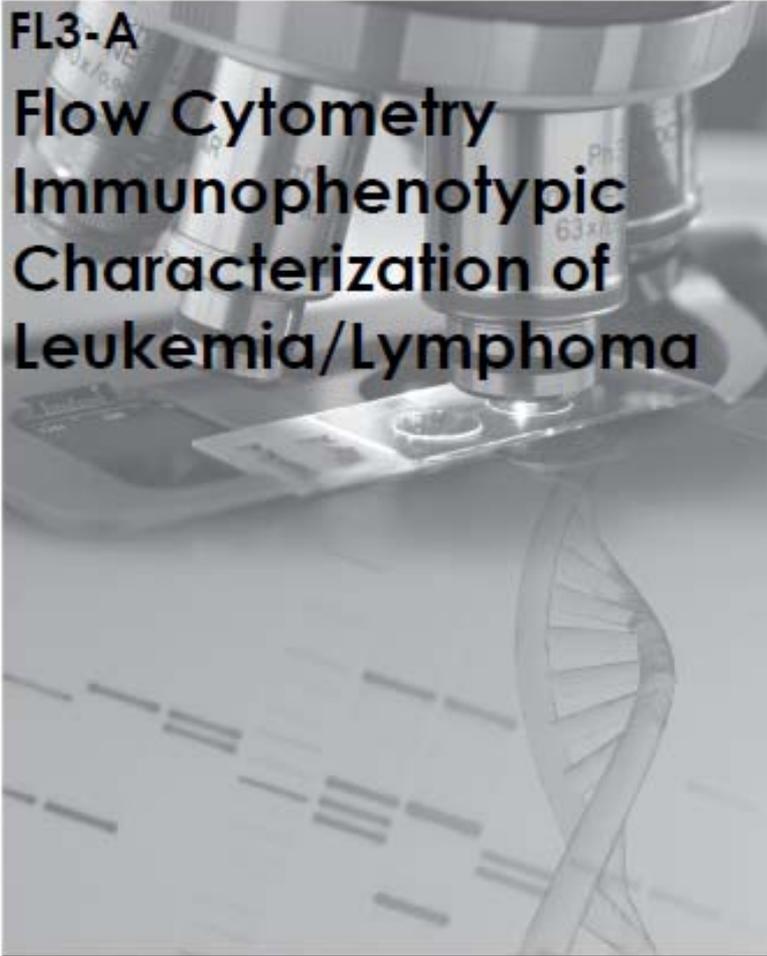
Other, specify:

030

Exception Code ⁰¹⁰ 11
 33

Favored Interpretation

⁰²⁰



FL3-A Flow Cytometry Immunophenotypic Characterization of Leukemia/Lymphoma

PARTICIPANT SUMMARY

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Preanalytic Characteristics

FL3-A 2012

Pre-Analytic Information

Viability

What percent of cells were viable?	FL3-01 Participant (505)		FL3-02 Participant (502)	
	No.	%	No.	%
0	2	0.4	2	0.4
10 – 20	4	0.8	3	0.6
21 – 30	3	0.6	2	0.4
31 – 40	4	0.8	4	0.8
41 – 50	3	0.6	5	1.0
51 – 60	5	1.0	7	1.4
61 – 70	9	1.8	3	0.6
71 – 80	11	2.2	12	2.4
81 – 90	60	11.9	23	4.6
91 – 95	123	24.4	76	15.1
96 – 99	243	48.1	306	61.0
100	38	7.5	59	11.8

Analytic Characteristics

- Viability Methods
- Gating Methods
- Use of Negative Control

Results

- Individual marker performance by platform
- Discussion of results with favored interpretation

Results Example

CD3

Software Antibody Distribution	Antibody Fluorescence Intensity					N	%
	DIM	BRI	HET	NOR	N/A		
BD FACSCalibur							
Negative	-	-	-	1	99	100	99.0
Partially Expressed	-	-	-	1	-	1	1.0
BD FACScan							
Negative	-	-	-	-	1	1	100.0
BD FACScanto							
Negative	-	-	-	-	17	17	100.0
BD FACScanto II							
Negative	-	-	-	-	132	132	100.0
BD FACSort							
Negative	-	-	-	-	2	2	100.0
Coulter Cytomics FC 500							
Negative	-	-	-	-	166	166	99.4
Positive	-	1	-	-	-	1	0.6
Coulter Epics XL (ALL models)							
Negative	-	-	-	-	14	14	100.0
Coulter Navios							
Negative	-	-	-	-	3	3	100.0
Other							
Negative	-	-	-	-	22	22	100.0
Totals	0	1	0	2	456	459	
% of Total	0.0	0.2	0.0	0.4	99.3		

FL3-01

Results Example

CD10

Software Antibody Distribution	Antibody Fluorescence Intensity						%
	DIM	BRI	HET	NOR	N/A	N	
BD FACSCalibur							
Negative	-	-	-	-	2	2	1.9
Positive	5	62	3	34	2	106	98.1
BD FACScan							
Positive	-	1	-	1	-	2	100.0
BD FACScanto							
Positive	-	16	-	4	-	20	100.0
BD FACScanto II							
Negative	-	-	-	-	1	1	0.7
Positive	3	109	1	29	3	145	98.6
Partially Expressed	1	-	-	-	-	1	0.7
BD FACSort							
Positive	-	1	-	-	1	2	100.0
Coulter Cytomics FC 500							
Positive	2	122	3	61	3	191	100.0
Coulter Epics XL (ALL models)							
Positive	1	12	1	3	-	17	100.0
Coulter Navios							
Positive	-	3	-	2	-	5	100.0
Other							
Positive	-	12	-	9	1	22	100.0
Totals	12	338	8	143	13	514	
% of Total	2.3	65.8	1.6	27.8	2.5		

Result Example

CD1a

Software Antibody Distribution	Antibody Fluorescence Intensity					N	%
	DIM	BRI	HET	NOR	N/A		
BD FACSCalibur Negative	-	-	-	1	7	8	100.0
BD FACScanto Negative	-	-	-	-	1	1	100.0
BD FACScanto II Negative	-	-	-	-	6	6	100.0
Coulter Cytomics FC 500 Negative	-	-	-	-	17	17	100.0
Coulter Epics XL (ALL models) Negative	-	-	-	-	2	2	100.0
Other Negative	-	-	-	-	2	2	100.0
Totals	0	0	0	1	35	36	
% of Total	0.0	0.0	0.0	2.8	97.2		

FL3-01

Result Example

Kappa Light Chain

Software Antibody Distribution	Antibody Fluorescence Intensity						N	%
	DIM	BRI	HET	NOR	N/A			
BD FACSCalibur								
Positive	2	66	4	32	1	105	99.1	
Partially Expressed	1	-	-	-	-	1	0.9	
BD FACScan								
Positive	-	2	-	-	-	2	100.0	
BD FACScanto								
Positive	-	17	-	3	-	20	100.0	
BD FACScanto II								
Positive	-	115	1	28	1	145	100.0	
BD FACSort								
Positive	-	1	-	-	1	2	100.0	
Coulter Cytomics FC 500								
Negative	-	-	-	-	1	1	0.5	
Positive	-	146	2	37	3	188	99.5	
Coulter Epics XL (ALL models)								
Positive	-	12	-	4	-	16	100.0	
Coulter Navios								
Positive	-	5	-	-	-	5	100.0	
Other								
Positive	-	17	-	5	-	22	100.0	
Totals	3	381	7	109	7	507		
% of Total	0.6	75.1	1.4	21.5	1.4			

FL3-01

Results Example

Favored Interpretation

	Participants (515)	
	No.	%
Burkitt lymphoma/leukemia (includes B-ALL)	452	87.8
CD10+ mature B lymphoid neoplasm, NOS	52	10.0
Diffuse large B-cell lymphoma	5	1.0
Precursor B-lymphoblastic leukemia/lymphoma	2	0.4
AML, favor acute myelomonocytic (M4) or monocytic (M5) leukemia	1	0.2
Hairy cell leukemia	1	0.2
Non-Hodgkin lymphoma, B-cell type, NOS	1	0.2
Insufficient information available for assessment	1	0.2

FL3-01

Discussion

B. Sample FL3-01 (Daudi cell line)

The clinical history was that of a 54-year-old male that presented with a one-week history of malaise, fatigue, and abdominal fullness. A CT scan of the abdomen revealed a large mass in the region of the right colon. Laboratory studies showed anemia and elevated lactate dehydrogenase (LDH) of 1,125 U/L [280-600 U/L]. A sample of the bone marrow was sent to the laboratory for flow cytometric immunophenotyping. The Wright-stained leukocytes revealed atypical lymphocytes with scant to moderate amount of cytoplasm and prominent cytoplasmic vacuoles.

The 80% consensus immunophenotype is a monoclonal B-cell population expressing CD10, CD19, CD20, CD22 (surface and cytoplasmic), CD38 (bright), CD45, CD71, CD79beta, HLA-DR, IgM, Kappa light chain (surface and cytoplasmic), cytoplasmic CD79alpha but not CD5. Other B-cell, T/NK-cell, myeloid and monocytic markers were absent (80% consensus) with the exception of a minority of laboratories reporting expression of CD23 (21%+) and CD33 (25%+).

The great majority (88%) of participants interpreted this case specifically as Burkitt lymphoma/leukemia (BL), and 10% of participants reported this case as CD10+ mature B-cell lymphoma, NOS. The clinical history (acute onset of disease with elevated LDH), morphology (prominent cytoplasmic vacuoles), and immunophenotype (CD10+, bright CD38 and CD71+) favor the diagnoses of BL. The bright CD38 expression, supporting the diagnosis of BL, further indicates the need of comparing the intensity of marker expression to normal counterparts as used in the current Survey. This is because CD38 expression is quite common in various B-cell lymphomas but bright expression is typical for BL. If the results were reported only as positive or negative without interpretation of intensity, the results of CD38 would not be able to support the BL diagnosis.

CD10+ mature B-cell lymphoma, NOS, is considered as acceptable interpretation, in particular if the participants were considering the possibility of B-cell lymphoma, unclassifiable (BCL-U) with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. BCL-U has many features overlapping with BL and further molecular genetic studies may be needed for differentiating these two entities. Whether the typical immunophenotype of BL is present in this new type of lymphoma (BCL-U) defined in the World Health Organization (WHO) 2008 classification remains to be determined.

Those participants choosing interpretations other than a Burkitt lymphoma or a mature CD10+ mature B-cell lymphoma, NOS, should review their immunophenotypic findings, gating strategies, and application of diagnostic criteria set forth in the 2008 WHO Classification.

FL3 Leukemia/Lymphoma

- Interpretation Summary 2008-2012
 - 20 challenges
 - Consensus (>80%) in 12/20
 - Correct response: Mean **91.3%** (SD 3.7%)
 - Non-consensus in 8/20
 - AML 5, Mature T-cell L/L 2, MCL 1
 - Acceptable responses **93.9%** (SD 6.7%)

FL3CD

- A survey for pathologists who use “technical only” flow cytometry and perform analysis of list mode files
 - A clinical history and images provided.
 - List mode files from anonymized cases are provided
 - Participants asked to analyze data and provide diagnostic interpretation
 - 120-140 participants

FL3CD-01: AML M4/M5

FL3CD-02: APL

FL3CD-01	Participant (144)	
	No.	%
AML, Favor Acute Myelomonocytic (M4) or Monocytic (M5) Leukemia	139	96.5
Acute Myeloid Leukemia (AML), NOS	3	2.1
AML, with (M1/M2) or without Maturation (M0)	1	0.7
Mantle Cell Lymphoma	1	0.7

FL3CD-02	Participant (144)	
	No.	%
AML, Favor Acute Promyelocytic Leukemia (M3)	111	77.1
Acute Myeloid Leukemia (AML), NOS	20	13.9
AML, Favor Acute Myelomonocytic (M4) or Monocytic (M5) Leukemia	5	3.5
AML, with (M1/M2) or without Maturation (M0)	5	3.5
AML, Favor Acute Erythroleukemia (M6)	2	1.4
Diffuse Large B-Cell Lymphoma	1	0.7

FL3CD performance summary

- 20 cases over 5 year period
- 10/20 cases had consensus
 - Mean % of intended responses = 91.9% (+/-5.5)
- 10/20 without consensus
 - Mean % w/ acceptable responses = 82.4% (+/- 14.3)
 - Range 49.5 – 99%

FL5 Survey

- Participants given clinical history and gated dot plots of anonymized cases
- Favored interpretation returned
- Started 2009
 - 30 participants initially
 - Now 80-90 participants

FL5 Survey

- 16 samples over 4 years
- 6 with consensus
 - Mean % intended response 91.6% (+/- 6.8)
- 10 without
 - Mean % intended response 72.1% (+/- 21.6)
- DIRC using challenging cases
 - Thymoma
 - Low level HCL with only lymph gate
 - Mis-gated sample

Other

- ZAP-70
 - Cell lines
- Use of stabilized primary human samples
 - PNH
- Movement toward stabilized primary human samples?