NEW TEST UPDATE
#1795 Leukemia/Lymphoma Flow EvaluatR™ available July 18, 2006

Dear Colleagues:

Specialty Laboratories is pleased to introduce a new, more informative Leukemia/Lymphoma panel which features several significant enhancements.

The Leukemia/Lymphoma Flow EvaluatR™ was created with the help of Specialty Laboratories/AmeriPath’s Hematopathology Resource Committee and key technical experts in flow cytometry. Working to standardize important diagnostic criteria, the Committee devised a panel that will ensure maximum sensitivity and specificity in the detection of aberrant cell populations—including secondary abnormalities which may be present in other cell lines such as in Myelodysplastic Syndromes (MDS)—and to standardize the interpretation of these abnormalities. The flow panel is modeled after requirements being defined by leading NIH consensus conference participants and is identical to the panel currently performed at AmeriPath’s six other flow cytometry centers located throughout the country.

Major improvements include:

1. Reporting changes to account for and more fully characterize all cell populations present including DNA ploidy and S-phase characteristics of these cells if contributory to the diagnosis and a morphologic summary, rather than just a list of markers on a single cell population.
2. New Specialty Tissue Transport Medium and collection requirements for enhanced cell viability and an optimized triaged set-up to identify cells of interest more rapidly and conserve them for ancillary studies.
3. Prioritization of markers performed to optimize diagnostic accuracy and avoid non-diagnostic workups.

In addition, expert technical analysts work with each pathologist on each case to eliminate analytical delays and provide rapid identification and triaging of samples for important ancillary tests such as FISH for t(15;17) in Acute Promyelocytic Leukemia.

Concurrently, our new panel introduces ZAP-70 as an ancillary marker for evaluating patients with newly diagnosed Chronic Lymphocytic Leukemia (CLL). ZAP-70 is performed via a state of the art methodology developed by our technical experts in flow cytometry (Shenkin M, Malese R., et al., Cytometry, in press) which significantly reduces assay variation related to stability of the sample.

To assist clients at all times, our flow cytometry panel is set up daily and on all weekend and holiday days, 365 days per year. We have also introduced additional flow cytometry instrumentation and expertly trained staff to increase capacity and redundancy. Experienced hematopathologists review each case and make detailed notes regarding key findings including a morphologic summary of cells of interest to correlate with the flow cytometry findings. All relevant data and interpretive comments are available to our interfaced clients directly from our LIS system at the time results are released.

Additionally, a full-color custom report (samples attached) incorporating selected images of cells and key histogram data will be sent to the client. The report includes key references from the medical literature pertinent to the established diagnosis. Finally, consultation with nationally recognized experts on test result interpretation is available daily.

Ordering information for the new panel is presented below.

<table>
<thead>
<tr>
<th>Test Order Information</th>
<th>Leukemia/Lymphoma Flow EvaluatR™ (#1795)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
<td>5.0 (0.3) mL Bone Marrow or Whole Blood in Sodium Heparin</td>
</tr>
<tr>
<td>Alternate Specimen 1</td>
<td>5.0 (0.3) mL ACD or EDTA Bone Marrow or Whole Blood</td>
</tr>
<tr>
<td>Stability</td>
<td>Refrigerated 30 hours</td>
</tr>
<tr>
<td>Collection Instructions</td>
<td>A smear should be submitted with Bone Marrow or Whole Blood specimens</td>
</tr>
<tr>
<td>Alternate Specimen 2</td>
<td>2.0 (0.5) g Tissue in Specialty Tissue Transport Medium</td>
</tr>
<tr>
<td>Stability</td>
<td>Refrigerated 30 hours</td>
</tr>
<tr>
<td>Alternate Specimen</td>
<td>Additional specimen types may be acceptable, contact Client Services.</td>
</tr>
<tr>
<td>Collection Instructions</td>
<td>Tissue, lymph node or spleen thinly sliced or minced in Specialty Tissue transport Medium. At least 0.5 g tissue, but more tissue may be necessary if there is fibrosis or low cellularity.</td>
</tr>
</tbody>
</table>
Leukemia/Lymphoma Flow EvaluatR™ (#1795)
July 2006, page 2 of 2

<table>
<thead>
<tr>
<th>Request</th>
<th>Specialty Tissue Transport Medium (TTM) from Specialty Client Supply</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shipping Instructions</td>
<td>Refrigerated samples are preferred. Ambient samples will be accepted; however, frozen specimens will be rejected. A specialized flow viability and count tube is used to evaluate sample acceptability for all samples received. Collect specimen in late afternoon for pick-up and delivery by overnight courier to arrive at Specialty within 24 hours of collection.</td>
</tr>
<tr>
<td>Clinical Utility</td>
<td>Immunophenotyping by flow cytometry distinguishes among various hematopoietic cell populations and determines the degree of expression of a panel of cell surface (as well as nuclear and cytoplasmic) antigens. Flow cytometry is used along with morphologic, cytogenetic, and molecular genetic analysis in the identification/classification of hematopoietic neoplasms (e.g., leukemia, lymphoma, myelodysplastic disorders).</td>
</tr>
<tr>
<td>Schedule</td>
<td>Daily</td>
</tr>
<tr>
<td>Reported</td>
<td>Same day received</td>
</tr>
<tr>
<td>CPT Codes</td>
<td>88182, 88184 [first marker], 88185 [per each additional marker], 88187, 88188, 88189</td>
</tr>
<tr>
<td>Notes</td>
<td>A basic panel of markers will be run on all specimens. Specialty Pathologists may select other antibody markers that in their medical judgment will provide crucial information related to a differential diagnosis. These tests are used to assess a variety of acute and chronic Leukemias and Lymphomas. If other markers are tested, an additional charge per marker will be added.</td>
</tr>
</tbody>
</table>

- If initial studies show increased blasts not clearly meeting WHO requirements for acute leukemia (AML≥20% blasts,), and there is insufficient clinical history, the referring oncologist and/or pathologist may be contacted by a Specialty Pathologist to obtain more detailed history before an interpretation is rendered. A Specialty Pathologist will contact the referring oncologist and/or pathologist or referring laboratory on all acute leukemias.  
- Large-cell lymphomas of both B- and T-cell lineage may not be detected by flow cytometry in some specimens because fragility of these cells leads to their destruction during processing of the specimen. 
- Flow cytometry may not resolve some T-cell leukemia/lymphomas if aberrant antigen expression is not present. 
- Flow cytometry is not useful for detecting Hodgkin’s disease. 
- Flow cytometry may detect increased percentages of myeloblasts, but may not be able to distinguish all low-grade myeloproliferative or myelodysplastic disorders and reactive processes. Additional ancillary testing such as cytogenetics, bcr/abl by FISH or RT-PCR, or JAK2 mutation analysis may be indicated in these circumstances. 

Please note: Our new flow cytometry panel incorporates significant service advantages over our previous panel, but it requires the full dedication of our expert staff to offer this test 365 days/year, hence the leukemia/lymphoma panels previously offered by Specialty will be replaced and will no longer be available shortly after the new Leukemia/Lymphoma Flow EvaluatR™ is activated. These tests include:  
1680 Leukemia/Lymphoma Evaluation Panel  
1681 FC Leukemia/Lymphoma Technical Only Panel  
1695 FC Blast/Acute Leukemia Panel  
1696 FC CLL/Lymphocyte Disorder Panel  

If you have any questions about our exciting new flow cytometry panel for leukemia/lymphoma or would like to know more about the advantages of Specialty’s Oncology and Hematology testing, please contact your Specialty Sales Representative or Client Services at 800-421-4449. You may also contact our Oncology Office directly at 661-799-6300 to speak with one of our Pathologists or a Medical Director.  

Michael C. Dugan, M.D.  
Vice President and Laboratory Director
Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma with kappa light chain restriction comprising 54% of all cells analyzed, CD38(+), ZAP-70(+).

Comment: Final interpretation requires correlation with clinical, morphologic, and the pending cytogenetic analysis findings. The results of the latter analysis will be issued in an addendum report. Should you have any questions, please contact me.

Morphology (Flow Cytometry Specimen)

The cytospin and smear preparations reveal an increased number of small lymphocytes.

Hematopathologist: [electronic signature] Christopher Lockhart, M.D.

Tests Performed


CPT Codes: 88184, 88185x24, 88189
Flow Cytometry Findings

Data were produced using correlated CD45 antigen density/right angle light scatter properties, gating on cell populations (approximate percentages of all cells analyzed) listed below:

ABNORMAL CELLS:
54% small sized (forward light scatter properties) monoclonal B cells with a kappa(+), CD45(+), CD5(+), CD19(+), CD20(+), CD23(+), HLA-DR(+), CD38(+) (91% of the clonal cells are positive), ZAP-70(+) (36% of the clonal cells are positive), CD10(-), FMC7(-), lambda(-) phenotype.

OTHER CELLS:
1) <1% Non-lymphoid blasts/progenitor cells [CD34(+), CD117(+), HLA DR(+)]
2) <1% Polytropic, predominantly small (forward light scatter properties) B cells [CD19(+), CD20(+), a kappa/lambda ratio = 1.5:1 and variable expression of CD22 and FMC7]
3) 12% Small T cells [CD3(+), CD4/CD8 ratio = 0.7:1, and non-aberrant expression of pan-T-cell antigens CD5 and CD7]
4) 3% NK cells [CD3(-), CD7(+), CD56 variable (+)]
5) 20% Granulocytic elements [CD13(+), CD16 variable(+), CD15(+)] without significant right angle light scatter property or antigenic expression pattern atypia
6) 3% Monocytes [CD11b(+), CD14(+), CD64(+)]

DNA: Non-contributory

SPECIMEN VIABILITY: 99%

FLOW CYTOMETRY DATA ANALYST: [electronic signature] Antonio Lazaro, CLS (CA)

REFERENCES:

The hematopathologist’s interpretation of these results should be considered a contributing portion of the physician’s workup. Correlation with all histologic and clinical data is necessary for a final interpretation. For questions about these results, please contact Client Services (800) 421-4449.

The performance characteristics of one or more of the assays in this panel were established through validation by Specialty Laboratories, and no approval is required by the U.S. Food and Drug Administration (FDA). These tests are used for clinical purposes. They should not be regarded as investigational or for research. Specialty Laboratories is regulated under the Clinical Laboratory improvement Amendments of 1988 (“CLIA”) as qualified to performed high complexity clinical testing.

Laboratory Director: [electronic signature] Michael Dugan, M.D.
Interpretation

The findings provide no immunophenotypic evidence of acute leukemia or a T-cell or B-cell neoplasm.

Comment: Final interpretation requires correlation with clinical and morphologic findings. Should you have any questions, please contact me.

Morphology (Flow Cytometry Specimen)

The cytospin and smear preparations reveal multilinear hematopoiesis with slight granulocytic left shift but no overt evidence of increased numbers of blasts. Apparent mild toxic changes are noted.
Markers Evaluated: CD3, CD4, CD5, CD7, CD8, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD23, CD34, CD45, CD56, CD64, CD117, FMC7, HLA-DR, Kappa, and Lambda

CPT Codes: 88184, 88185x21, 88189

Flow Cytometry Findings

Data was produced using correlated CD45 antigen density/right angle light scatter properties, gating on cell populations (approximate percentages of all cells analyzed) listed below:

ABNORMAL CELLS: None Detected

OTHER CELLS:
1) <1% Non-lymphoid blasts/progenitor cells [CD34(+), CD117(+), HLA DR(+)]
2) 5% Polytypic, predominantly small (forward light scatter properties) B cells [CD19(+), CD20(+) with a kappa/lambda ratio = 1.5:1 and variable expression of CD23 and FMC7]
3) 8% Small T cells [CD3(+), CD4/CD8 ratio = 0.9:1, and non-aberrant expression of pan-T-cell antigens CD5 and CD7]
4) 1% NK cells [CD3(-), CD7(+), CD56 variable(+)]
5) 73% Granulocytic elements [CD13(+), CD16 variable(+), CD15(+)] with changes of slight left shift
6) 5% Monocytes [CD11b(+), CD14(+), CD64(+)]
7) Erythroid cells/CD45(-) cells/debris comprise the majority of the remaining events.

DNA: Non-contributory

SPECIMEN VIABILITY: 99%

FLOW CYTOMETRY DATA ANALYST: [electronic signature] Antonio Lazaro, CLS (CA)

REFERENCES:

The hematopathologist’s interpretation of these results should be considered a contributing portion of the physician’s workup. Correlation with all histologic and clinical data is necessary for a final interpretation. For questions about these results, please contact Client Services (800) 421-4449.

The performance characteristics of one or more of the assays in this panel were established through validation by Specialty Laboratories, and no approval is required by the U.S. Food and Drug Administration (FDA). These tests are used for clinical purposes. They should not be regarded as investigational or for research. Specialty Laboratories is regulated under the Clinical Laboratory improvement Amendments of 1988 (“CLIA”) as qualified to performed high complexity clinical testing.

Laboratory Director: [electronic signature] Michael Dugan, M.D.