SPECIALTY LABORATORIES
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July 2006

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, Maiese 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.

Test Order Information	Leukemia/Lymphoma Flow EvaluatR™ (#1795)
Specimen	5.0 (3.0) mL Bone Marrow or Whole Blood in Sodium Heparin
Alternate Specimen 1	5.0 (3.0) mL ACD or EDTA Bone Marrow or Whole Blood
Stability	Refrigerated 30 hours
Collection Instructions	A smear should be submitted with Bone Marrow or Whole Blood specimens
Alternate Specimen 2	2.0 (0.5) g Tissue in *Specialty* Tissue Transport Medium
Stability	Refrigerated 30 hours
Alternate Specimen	Additional specimen types may be acceptable, contact Client Services.
Collection Instructions	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.

	Request *Specialty* Tissue Transport Medium (TTM) from *Specialty* Client Supply
Shipping Instructions	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.
Clinical Utility	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).
Schedule	Daily
Reported	Same day received
CPT Codes	88182, 88184 [first marker], 88185 [per each additional marker], 88187, 88188, 88189
Notes	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.

- 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

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Account # 1234

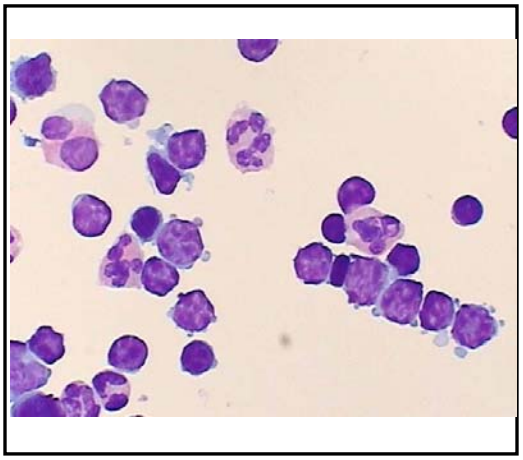
FINAL CHARTABLE REPORT

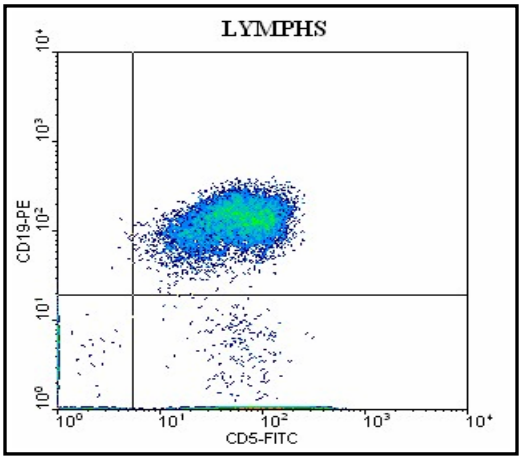
Patient:	**SMITH, JANE**	
Sex:	Female	SSN: 123-98-7654
Date of Birth:	03/06/1946	Age: 60 Years
Patient ID:	A0123456799	
Physician:	Doe, Jane	
Collection Date:	06/06/2006	
Specimen:	Bone Marrow	

Client Specimen ID	Specialty Accession #
01234567:ABC	006 - 1234566
Received Date	**Report Date**
06/07/2006	06/08/2006

Notes:

1795 – Leukemia / Lymphoma Flow EvaluatR ™





Microscopic image(s) and/or histogram(s) are a symbolic representation of key findings of this specific report and are not intended to replace a complete review and reading of the final diagnostic report provided.

Interpretation

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

Markers Evaluated: CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, Mo2, CD15, CD16, CD19, CD20, CD23, CD34, CD38, CD45, CD56, CD64, CD117, FMC7, HLA-DR, Kappa, Lambda, and ZAP-70.

CPT Codes: 88184, 88185x24, 88189

Report Continued On Next Page

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Patient: SMITH, JANE

Specialty Accession # 006 - 1234566

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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% 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) 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:
1) Braylan, R. C., Borowitz, M. J., Davis, B. H., Stelzer, G. T., Stewart, C. C., et al. 1997. U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry. Cytometry 30, no. 5: 213-63.
2) Stelzer, G. T., Shults, K. E., and Loken, M. R. 1993. CD45 Gating for Routine Flow Cytometric Analysis of Human Bone Marrow Specimens. Annals of the New York Academy of Sciences 677: 265-80.
3) Jaffe E.S., Harris N.L., Stein H., Vardiman J.W., eds. 2001. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues (ISBN 92-832-2411-6). Lyon: IARC Press.
4) Wiestner, A., Rosenwald, A., Barry, T., et al. 2003. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 101(12):4944-51.
5) Crespo, M., Bosch, F., Villamoor, N. et al. 2003. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. New England Journal of Medicine 348:1764-75.

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.

Report Completed

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Fax: (555) 555-5556

Account # 1234

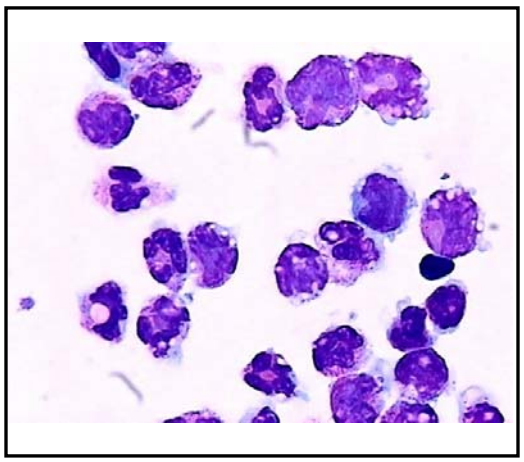
Patient:	**SMITH, JOHN**	
Sex:	Male	SSN: 123-45-6789
Date of Birth:	02/29/1926	Age: 80 Years
Patient ID:	A0123456789	
Physician:	Doe, Jane	
Collection Date:	06/06/2006	
Specimen:	Bone Marrow	

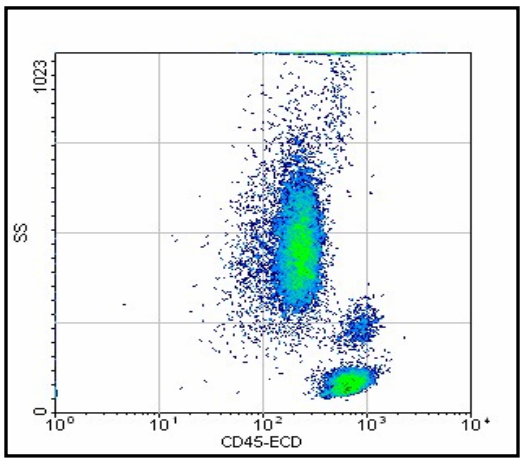
Client Specimen ID	Specialty Accession #
01234567:ABC	006 - 1234567
Received Date	**Report Date**
06/07/2006	06/08/2006

Notes:

FINAL CHARTABLE REPORT

1795 – Leukemia / Lymphoma Flow EvaluatR ™





Microscopic image(s) and/or histogram(s) are a symbolic representation of key findings of this specific report and are not intended to replace a complete review and reading of the final diagnostic report provided.

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.

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

Report Continued On Next Page

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Patient: SMITH, JOHN

Specialty Accession # 006 - 1234567

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Tests Performed

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:
1) Braylan RC, Borowitz MJ, Davis BH, et.al. 1997, U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry. Cytometry 30; No.5; 213-63.

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.

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Laboratory Director: *[electronic signature]*

Michael Dugan, M.D.

Report Completed