Immunophenotyping Utilizing 6 Color Flow Cytometry

John L. Carey, M.D.

INTRODUCTION / RATIONAL

Beginning on December 5, 2011, the Warde Medical laboratory’s flow cytometry section changed from a 4-to a 6-color analysis for evaluating for leukemias and lymphomas. This now allows for the full utilization of the new generation of flow cytometers acquired over the last several years. The major advantages associated with this change are

• Fewer tubes, hence faster analytic throughput & greater labor efficiency,
• Fewer duplicate (non-billable) antigens in a panel and
• more modern alignment with current recommendation for flow immunophenotyping of hematolymphoid neoplasias.

CHANGES

1. Mature B Lineage Neoplasias

B Lymphoid

The current panel content for B cell clonality (Order code SHORTBCELL), Mature B cell lymphoma/leukemias (order code BCELL) and for Hairy Cell leukemia (order code HAIRYCELL) are unchanged in content. However, the number of tubes are essentially cut in half, with a significant reduction in duplicate antigens. The increased combination of simultaneously analyzed antigens allows for a more sensitive evaluation of minor B cell monoclones, as well as clearer identification of major lymphoid subsets (eg, T, B, NK, see Figure 1).
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Plasma Cell Dyscrasias
Flow analysis for plasma cell dyscrasia is not routinely needed for diagnosis — morphologic exam of the marrow along with serum & urine protein electrophoresis/immunofixation are typically sufficient.

However, flow analysis can be a particularly useful application in the setting of a known minor monoclonal gammopathy (< 3.0 g/dL) when there are relatively few plasma cells present in the morphologic specimen. The new plasma cell panel is designed to efficiently identify a small fraction of plasma cells and ascertain if they are monoclonal, polyclonal or a mixture of both — all in a single tube (order code MYELOMA).

2. Mature T Lymphoid Neoplasias

Mature T Cell Panel
Similar to the B lymphoid panels, the mature T cell lymphoma/leukemia panel (order code TCELL) has reduced by half the number of tubes needed, while allowing for a greater ease of directly visualizing coexpressed T and NK associated antigens (see Figure 2). In order to accommodate

Table 1. Summary of Immunophenotyping Panels Available by 6-Color Flow Cytometry

<table>
<thead>
<tr>
<th>Immunophenotype Panel:</th>
<th>Test Code:</th>
<th>Markers Performed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature Leukemia/Lymphoma, Probably B-cell</td>
<td>BCELL</td>
<td>CD3, 5, 10, 11c, 19, 20, 23, 38, 45, Kappa &amp; Lambda</td>
</tr>
<tr>
<td>Mature Leukemia/Lymphoma, Probably T-cell</td>
<td>TCELL</td>
<td>CD2, 3, 4, 5, 7, 8, 16+56, 25, 26, 45, 56 &amp; 57</td>
</tr>
<tr>
<td>B Cell Clonality Only</td>
<td>SHORTBCELL</td>
<td>CD5, 19, 23, 45, Kappa &amp; Lambda</td>
</tr>
<tr>
<td>Acute Lymphoblastic or Myeloid Leukemia</td>
<td>ACUTE</td>
<td>CD2, 3, 5, 7, 10, 11b, 13, 14, 15, 16, 19, 20, 33, 34, 45, 56, 61, 117, 235a &amp; HLA-Dr</td>
</tr>
<tr>
<td>Plasma Cell/Myeloma</td>
<td>MYELOMA</td>
<td>CD19, 56, 45, 38, cytoKappa &amp; cytoLambda</td>
</tr>
<tr>
<td>Hairy Cell Leukemia</td>
<td>HAIRYCELL</td>
<td>CD3, 5, 10, 11c, 19, 20, 22, 23, 25, 38, 45, 103, Kappa &amp; Lambda</td>
</tr>
<tr>
<td>Sezary Staging</td>
<td>SEZ</td>
<td>CD3, 4, 7, 8, 26 &amp; 45</td>
</tr>
<tr>
<td>Acute Leukemia in cerebrospinal fluid</td>
<td>ACUTECSSF</td>
<td>CD19, 13+33, 34, 45, cytoTdT &amp; cytoCD3</td>
</tr>
<tr>
<td>Fluid T Cell Subsets (Bronchoalveolar or other non-blood fluids)</td>
<td>BAL348</td>
<td>CD3, 4, 8, 45</td>
</tr>
</tbody>
</table>
a new T related antigen (CD26) and/or substitute in other T related antigens (eg, CD10 with a differential of angioimmunoblastic T NHL), the NK antigens CD16 and CD56 are combined. A follow up analysis separating these two antigens can be performed if desired.

Sezary Staging Panel

Tube #1 of the T cell panel can be used as a stand-alone staging analysis for involvement of the blood by a Cutaneous T cell lymphoma (ordercode SEZ). This evaluates for immunophenotypic abnormalities associated with these types of T cell neoplasias (eg, excess CD4+ T cells; loss of CD7 or CD26 on the CD4+ T cells). It is compliant with international staging protocols for cutaneous T cell lymphomas and Sezary syndrome.

3. Acute Leukemia & MDS

The new acute leukemia panel (order code ACUTE) is designed to more fully evaluate for acute leukemia as well as other causes of marrow failure and associated cytopenias (eg, myelodysplasia; B lymphoproliferative disorders). This includes a fuller ability to evaluate for myeloid maturation abnormalities, hematogones hyperplasia vs. precursor B lymphoblastic leukemia and a more direct visualization of atypical antigen expression on both mature B and T lymphs (see Figure 3). Lastly, if desired, an evaluation of cytoplasmic antigens to define major lineage (T vs. B vs. myeloid) is available as a single tube addition to the standard acute leukemia panel (cyto. TdT/cyto. MPO/ cyto. CD3/cyto. CD79a).

4. CSF Fluids

Flow immunophenotypic analysis of CSF for leukemia or lymphoma is really only indicated in patients with (1) a past medical / laboratory history of leukemia or lymphoma or (2) highly suggestive imaging or CSF cytologic evidence hematolymphoid neoplasia (eg, elevated or atypical lymphs; blasts).

In the usual clinical setting in North America, the major differential diagnoses will be between a mature B cell neoplasm and an acute leukemia. As such, there are two new panels — each a single tube — to evaluate for one or the other. The B cell clonality (order code SHORTBCSF) panel is the same as for non-CSF samples, and allows identification of major lymphoid subsets and B cell clonality and differentiation in one tube.

In the setting of suspected acute leukemia, a single tube panel designed to detect T & B lymphoblastic and AML is used (order code ACUTE CSF), using a mixture of membrane and cytoplasmic antigens. The specificity of the final diagnosis will be critically dependent upon correlation with other clinical and lab data.

Lastly, CSF samples reasonably submitted for flow analysis should be immediately processed and submitted in a 1:1 dilution of tissue media (eg, RPMI; McCoy’s).
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Figure 1: B cell Analysis
Figure 2: T cell Analysis
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Figure 3: Acute Leukemia/MDS panel:

Simultaneous Multi-population Analysis of Major Marrow Lineages

Myeloid Maturation Analysis for MDS Evaluation

Precursor B cell (lymphoblast) Analysis: Hematogone Hyperplasia
Editorial: Harnessing the Power of Multicolor Flow Cytometry

William G. Finn, M.D.

In this issue of the Warde Report, Dr. John Carey, scientific director of flow cytometry at Warde Medical Laboratory, provides details of the changes involved in our recent deployment of 6-color flow cytometry. This enhancement is likely to bring greater value—both economic and medical—to Warde laboratory clients and co-tenants. The expansion of polychromatic flow cytometry platforms does more than simply reduce the number of assays (“tubes”) necessary to run a given analysis. It also optimizes the ability to isolate specific cell types, both in reactive and neoplastic diseases, that are defined by specific simultaneous combinations of multiple markers rather than by hierarchical lists of expressed antigens.

Global patterns of marker expression, including expected sequences of marker acquisition based on higher dimensional analysis, are more important than simply the list of markers expressed by a given cell type. In polychromatic flow cytometry, each measured parameter is analogous to a spatial coordinate, and the aggregate of many parameters forms in essence a high dimensional “shape.” The familiar histograms of a flow cytometric analysis are effectively 2-dimensional projections of a higher dimensional space. Just as we gain more information by walking through a 3-dimensional room than we do by examining a 2-dimensional snapshot of that room, we gain more information about cell populations when we can characterize them based on aggregate phenotypes of numerous markers, analyzable from many different vantage points in a higher dimensional analysis.

For many years, flow cytometry has been a valuable tool in the detection of abnormal cell populations, often in very low numbers, in the background of normal cellular constituents. This function can be enhanced when panels are designed around not only the likely characteristics of the abnormal cells being targeted, but also around the normal cellular constituents of a given sample. For instance, panels capable of discerning the maturation patterns of normal B-lymphocyte precursors (hematogones) in bone marrow may more effectively enable the interpreter to detect abnormal B-lymphoblasts in acute lymphoblastic leukemia. Likewise, the ability to isolate normal germinal center B-lymphocytes in a lymph node sample may allow for a more sensitive detection of partial lymph node involvement by lymphoma. Similar approaches allow distinction of normal from leukemic myeloblasts, and so on. These types of scenarios are better enabled by more available fluorescence channels (colors) that allow a fuller immunophenotyping of normal maturation patterns based on aggregate marker combination patterns.
Editorial: Harnessing the Power of Multicolor Flow Cytometry

Polychromatic flow cytometry (10-color and beyond) has been used in the research setting for some time, but the diagnostic utility of polychromatic flow cytometry is likely to expand as clinical laboratories gain more experience with higher dimensional analysis in clinical-grade instruments. For instance, the use of flow cytometry in the diagnosis of myelodysplastic syndromes — now somewhat controversial — is likely to become more mainstream as more laboratories deploy higher dimensional analyses allowing for standardization of myeloid maturation patterns in healthy and diseased bone marrow.

In the era of genomics and personalized medicine, the continued development of more and more “colors” in diagnostic flow cytometry is resulting in the transition of this technology toward an iterative, cytomic discovery tool that will allow us to keep pace with advances in diagnostic classification and therapeutic monitoring.

References.