Overview

Useful For
Evaluating lymphocytoses of undetermined etiology
Identifying B- and T-cell lymphoproliferative disorders involving blood and bone marrow
Distinguishing acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML)
Immunologic subtyping of ALL
Distinguishing reactive lymphocytes and lymphoid hyperplasia from malignant lymphoma
Distinguishing between malignant lymphoma and acute leukemia
Phenotypic subclassification of B- and T-cell chronic lymphoproliferative disorders, including chronic lymphocytic leukemia, mantle cell lymphoma, and hairy cell leukemia
Recognizing AML with minimal morphologic or cytochemical evidence of differentiation
Recognizing monoclonal plasma cells

Reflex Tests

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Additional Tests

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The testing process begins with a screening panel. The screening panel will be charged based on the number of markers tested (FIRST for first marker, ADD1 for each additional marker). The interpretation will be based on markers tested in increments of 2 to 8, 9 to 15, or 16 and greater. In addition, reflex testing may occur to fully characterize a disease state or clarify any abnormalities from the screening test. Reflex tests will be performed at an additional charge for each marker tested (FIRST if applicable, ADD1 if applicable).

In addition to reflexing flow cytometric panels, AML FISH testing for PML-RARA translocation t(15;17) may be added by the Mayo Clinic pathologist to exclude acute promyelocytic leukemia if there is morphologic suspicion or if blasts and promyelocytes are CD34-negative and HLA-DR-negative.

The triage panel is initially performed to evaluate for monotypic B cells by kappa and lambda light chain expression, increased numbers of blast cells by CD34 and CD45 expression along with side scatter gating, and increased plasma cells by CD45 expression and side scatter gating. The triage panel also includes antibodies to assess the number of CD3-positive T cells and CD16-positive/CD3-negative natural killer (NK) cells present. This triage panel also determines if there is an increase in the number of T cells that aberrantly coexpress CD16, an immunophenotypic feature of T-cell granular lymphocytic leukemia.

This panel, together with the provided clinical history and morphologic review, is used to determine what, if any, additional testing is needed for disease diagnosis or classification. If additional testing is required, it will be added per the algorithm to fully characterize a disease state with a charge per unique antibody tested.

If no abnormalities are detected by the initial panel, no further flow cytometric assessment will be performed unless otherwise indicated by specific features of the clinical presentation or prior laboratory results.

Additional FISH or molecular testing may be recommended by the Mayo pathologist to facilitate diagnosis. The referring physician or pathologist will be contacted to confirm the addition of any of these tests. Cytogenetic FISH Studies:

- **CCND1/IGH translocation t(11;14)**, to exclude mantle cell lymphoma in cases of CD5+CD23- B-cell lymphoproliferative disorder.

- **TCL-1 break-apart at 14q32**, to exclude T-cell prolymphocytic leukemia in cases with CD4-positive T-cell lymphoproliferative disorder (phenotypic aberrancy or very tight CD4+ population with high CD4:CD8 ratio).

- **MYC break-apart at 8q24**, with or without **IGH-BCL2 t(14;18) and BCL6** break-apart at 3q27, for suspected high grade B-cell lymphomas, based on morphologic assessment and immunophenotype (usually CD10-positive).

Molecular Genetic Studies:

- T-cell receptor gene rearrangement to examine clonality of T cells in cases showing phenotypically aberrant T-cell population.

Cytochemical Stains:

- Confirmatory cytochemical stains as needed.

The following algorithms are available in Special Instructions:

- **Bone Marrow Staging for Known or Suspected Malignant Lymphoma Algorithm**

- **Acute Myeloid Leukemia: Testing Algorithm**
Method Name
Immunophenotyping

NY State Available
Yes

Specimen

Specimen Type
Varies

Advisory Information
This test is appropriate for hematopoietic specimens only. For solid tissue specimens, order LLPT / Leukemia/Lymphoma Immunophenotyping by Flow Cytometry, Tissue.

Bone marrow specimens being evaluated for possible involvement by a myelodysplastic syndrome (MDS) or a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) including chronic myelomonocytic leukemia (CMML) should be ordered as MYEFL / Myelodysplastic Syndrome by Flow Cytometry, Bone Marrow.

Bronchoalveolar lavage specimens submitted for evaluation for leukemia or lymphoma are appropriate to send for this test.

This test is not appropriate for and cannot support diagnosis of sarcoidosis, hypersensitivity pneumonitis, interstitial lung diseases, or differentiating between pulmonary tuberculosis and sarcoidosis (requests for CD4/CD8 ratios); specimens sent for these purposes will be rejected.

If cytogenetic tests are also desired when drawing specimen for this test, an additional specimen should be submitted. It is important that the specimen be obtained, processed, and transported according to instructions for the other required test.

Shipping Instructions
Specimen must arrive within 48 hours of collection for spinal fluid, 72 hours for fluids, or 96 hours for peripheral blood and bone marrow.

Necessary Information
The following information is required:

1. Pertinent clinical history including reason for referral or clinical indication
2. Clinical or morphologic suspicion

3. Specimen source

4. Date and time of collection

5. For spinal fluid specimens: spinal fluid cell and differential counts are required.

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Blood

Container/Tube:

Preferred: Yellow top (ACD solution A or B)

Acceptable: Sodium heparin, EDTA

Specimen Volume: 6 mL

Slides: Include 5 to 10 unstained blood smears, if possible.

Collection Instructions:
1. Do not transfer blood to other containers.

2. Label specimen as blood.

Specimen Stability Information: Ambient <96 hours/Refrigerated < or =96 hours

Specimen Type: Bone marrow

Container/Tube:

Preferred: Yellow top (ACD solution A or B)

Acceptable: Sodium heparin, EDTA

Specimen Volume: 1-5 mL

Slides: Include 5 to 10 unstained bone marrow aspirate smears, if possible.

Collection Instructions:
1. Submission of bilateral specimens is not required.

2. Label specimen as bone marrow.

Specimen Stability Information: Ambient <96 hours/Refrigerated < or =96 hours
Specimen Type: Fluid

Sources: Serous effusions, pleural fluid, pericardial fluid, abdominal (peritoneal) fluid

Container/Tube: Body fluid container

Specimen Volume: 20 mL

Collection Instructions:

1. If possible, fluids other than spinal fluid should be anticoagulated with heparin (1 U/mL of fluid).

2. The volume of fluid necessary to phenotype the lymphocytes or blasts in serous effusions depends upon the cell count in the specimen. Usually 20 mL of pleural or peritoneal fluid is sufficient. Smaller volumes can be used if there is a high cell count.

3. Label specimen with fluid type.

Specimen Stability Information: Refrigerated <72 hours/Ambient < or =72 hours

Specimen Type: Spinal fluid

Container/Tube: Sterile vial

Specimen Volume: 1-1.5 mL

Collection Instructions:

1. An original cytospin preparation (preferably unstained) must be included with the spinal fluid specimen so correlative morphologic evaluation can occur.

2. The volume of fluid necessary to phenotype the lymphocytes or blasts in spinal fluid depends upon the cell count in the specimen. A cell count should be determined and submitted with the specimen. Usually 1 to 1.5 mL of spinal fluid is sufficient. Smaller volumes can be used if there is a high cell count. If cell count is <10 cells/mcL, a larger volume of spinal fluid may be required. When cell counts drop below 5 cells/mcL, the immunophenotypic analysis may not be successful.

3. Label specimen as spinal fluid.

Specimen Stability Information: Refrigerated <48 hours/Ambient < or =48 hours

Forms

1. Hematopathology Patient Information (T676) in Special Instructions

2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

   - Hematopathology/Cytogenetics Test Request (T726)

   - Benign Hematology Test Request (T755)

Specimen Minimum Volume
Blood: 3 mL
Bone Marrow, Spinal Fluid: 1 mL
Fluid from Serous Effusions: 5 mL

Reject Due To

| Gross hemolysis | Reject |

Specimen Stability Information

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Clinical and Interpretive

Clinical Information
Diagnostic hematopathology has become an increasingly complex subspecialty, particularly with neoplastic disorders of blood and bone marrow. While morphologic assessment of blood smears, bone marrow smears, and tissue sections remains the cornerstone of lymphoma and leukemia diagnosis and classification, immunophenotyping is a very valuable and important complementary tool.

Immunophenotyping hematopoietic specimens can help resolve many differential diagnostic problems posed by the clinical or morphologic features.

Reference Values
An interpretive report will be provided.

This test will be processed as a laboratory consultation. An interpretation of the immunophenotypic findings and correlation with the morphologic features will be provided by a hematopathologist for every case.

Interpretation
Report will include a morphologic description, a summary of the procedure, the percent positivity of selected antigens, and an interpretive conclusion based on the correlation of the clinical history with the morphologic features and immunophenotypic results.

Cautions
Specimens will be initially triaged to determine which, if any, of the immunophenotyping panels should be performed.

Clinical Reference

5. Hoffman RA, Kung PC, Hansen QP, Goldstein G: Simple and rapid measurement of T lymphocytes and their subclass in peripheral blood. Proc Natl Acad Sci USA 1980;77:4914-4917


Performance

Method Description
Flow cytometric immunophenotyping of peripheral blood, bone marrow, and body fluids is performed using the following antibodies:

Triage Panel: CD3, CD10, CD16, CD19, CD34, CD45 and kappa and lambda light chains

Possible Additional Panels:

-B-cell Panel: CD5, CD11c, CD19, CD20, CD22, CD23, CD38, CD45, CD103, CD200 and kappa and lambda light chains

-T-cell Panel: CD2, CD3, CD4, CD5, CD7, CD8, CD45, and gamma/delta

-Killer-cell immunoglobulin-like receptor (KIR) Panel: CD3, CD8, CD16, CD56, CD57, CD94, CD158a, CD158b, CD158e (p70), and NKG2a

-V-Beta Panel: CD3, CD8, T-cell receptors: VB1, VB2, VB3, VB4, VB5.1, VB5.2, VB5.3, VB7.1, VB7.2, VB8, VB9, VB11, VB12, VB13.1, VB13.2, VB13.6, VB14, VB16, VB17, VB18, VB20, VB21.3, VB22, and VB23

-Acute Panel: CD2, CD7, CD13, CD15, CD16, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, and HLA-DR

-B-cell ALL, minimal residual disease (MRD) panel: CD9, CD10, CD19, CD20, CD34, CD38, CD45, CD66c

-Myeloperoxidase (MPO)/terminal deoxynucleotidyl transferase (TdT) (MPO/TdT) Panel: cytoplasmic CD3, CD13, cytoplasmic CD22, CD34, CD45, cytoplasmic CD79a, nuclear TdT, and cytoplasmic MPO

-Plasma Cell Panel: CD19, CD38, cytoplasmic kappa and lambda light chains

-Mast Cell Panel: CD2, CD25, CD69, CD117

PDF Report
No

Day(s) and Time(s) Test Performed
Specimens are processed and reported Monday through Saturday

Analytic Time
1 day

Maximum Laboratory Time
4 days

Specimen Retention Time
Remaining blood/bone marrow, 14 days; Remaining fluid, 7 days

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
88184-Flow cytometry; first cell surface, cytoplasmic or nuclear marker x 1

88185-Flow cytometry; additional cell surface, cytoplasmic or nuclear marker (each)

88187-Flow Cytometry Interpretation, 2 to 8 Markers (if appropriate)

88188-Flow Cytometry Interpretation, 9 to 15 Markers (if appropriate)

88189-Flow Cytometry Interpretation, 16 or More Markers (if appropriate)

LOINC® Information

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