Overview

Useful For

Evaluating lymphocytoses of undetermined etiology

Identifying B- and T-cell lymphoproliferative disorders involving blood and bone marrow

Distinguishing acute lymphoblastic leukemia from acute myeloid leukemia (AML)

Immunologic subtyping of acute leukemias

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

Test ID	Reporting Name	Available Separately	Always Performed
VBETA	TCR V-BETA	No	No
FCINT	Flow Cytometry Interp, 2-8 Markers	No, (Bill Only)	No
FCIMS	Flow Cytometry Interp, 9-15 Markers	No, (Bill Only)	No
FCINS	Flow Cytometry Interp,16 or greater	No, (Bill Only)	No

Additional Tests

Test ID	Reporting Name	Available Separately	Always Performed
FIRST	Flow Cytometry, Cell Surface, First	No, (Bill Only)	Yes
ADD1	Flow Cytometry, Cell Surface, Addl	No, (Bill Only)	Yes

Testing Algorithm

Note: This test is only available to clients who have MayoAccess or MayoLink.

The client is responsible for the interpretation and billing of the professional component; Mayo Clinic will bill the technical component only.

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). 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 (ADD1 if applicable).

The triage panel is initially performed on peripheral blood, bone marrow, and fluid samples to evaluate for monotypic B cells by kappa and lambda light chain expression, increased numbers of blasts by CD34 and CD45 expression along with side scatter gating, and increased plasma cells by CD45 expression with 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.

The tissue panel is initially performed to evaluate for monotypic B-cells by kappa and lambda light chain expression, increased numbers of blasts, and plasma cells by CD45 expression along with side scatter gating. The panel can also evaluate T cells with CD3, CD5, and CD7. Additionally, viability is assessed on all tissue specimens using 7-AAD exclusion.

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

Cases requiring the granular lymphocytic leukemia flow panel or V-beta panel will have an interpretation added and performed by a Mayo Clinic pathologist.

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.

The following algorithms are available in Special Instructions:

-Bone Marrow Staging for Known or Suspected Malignant Lymphoma Algorithm

-Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up

Special Instructions

- Hematopathology Patient Information
- Bone Marrow Staging for Known or Suspected Malignant Lymphoma Algorithm
- Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up

Method Name

Immunophenotyping

NY State Available

Yes

Specimen

Specimen Type

Varies

Advisory Information

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 (BAL), bronchial washings, and lung tissue specimens are **not acceptable** for this test due to the highly contagious nature of COVID-19 that could be present. The use of immunohistochemical (IHC) stains is encouraged for immunophenotyping in these specimens.

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.**

Additional Testing Requirements

For bone marrow testing, if cytogenetic tests are desired along with this test request, an additional specimen should be submitted. It is important that the specimen be obtained, processed, and transported according to instructions for the other test.

Shipping Instructions

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

Necessary Information

1. The following information is required:

a. Pertinent clinical history including reason for testing or clinical indication

b. Clinical or morphologic suspicion

c. Specimen source

d. Date and time of collection

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

f. For tissue specimens: tissue type and location are required.

2. **A pathology/diagnostic report including the client surgical pathology case number, a brief history, reason for referral or clinical suspicion are required** before the specimen will be processed.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Blood

Container/Tube:

Preferred: Yellow top (ACD solution A or B)

Acceptable: Green top (sodium heparin) or lavender top (EDTA)

Specimen Volume: 10 mL

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

Collection Instructions:

1. Send specimen in original tube. 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: Green top (sodium heparin) or lavender top (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, pericardial, or 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

Specimen Type: Tissue

Supplies: Hank's Solution (T132)

Container/Tube: Sterile container with 15 mL of tissue culture medium (eg, Hank's balanced salt solution [T132], RPMI, or equivalent)

Specimen Volume: 5 mm(3) or larger biopsy

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

Collection Instructions:

1. Send intact specimen **(do not mince)**.

2. Specimen cannot be fixed.

Forms

1. Hematopathology Patient Information (T676) in Special Instructions

2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Blood: 3 mL
Bone Marrow, Spinal Fluid: 1 mL
Fluid from Serous Effusions: 5 mL
Tissue: 1 mm(3) or larger biopsy

Reject Due To

Gross hemolysis	Reject
Bronchoalveolar lavage (BAL) or bronchial washings, lung tissue, paraffin-embedded tissue	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

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. This test is appropriate for hematopoietic specimens only.

This is a technical only test and does not include interpretation unless reflex testing is performed. At any point, clients may request to have a Mayo Clinic hematopathologist provide an interpretation at an additional charge.

Reference Values

Not applicable

Interpretation

Report will include a summary of the procedure.

Cautions

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

Clinical Reference

1. Hanson CA, Kurtin PJ, Katzman JA, et al: Immunophenotypic analysis of peripheral blood and bone marrow in the staging of B-cell malignant lymphoma. Blood 1999;94:3889-3896

2. Hanson CA: Acute leukemias and myelodysplastic syndromes. In Clinical Laboratory Medicine. Edited by KD McClatchey. Williams and Wilkins, Inc, 1994, pp 939-969

3. Morice WG, Leibson PJ, Tefferi A: Natural killer cells and the syndrome of chronic natural killer cell lymphocytosis. Leuk Lymphoma 2001;41(3-4):277-284

4. Langerak AW, van Den Beemd R, Wolvers-Tettero IL, et al: Molecular and flow cytometric analysis of the Vbeta repertoire for clonality assessment in mature TCR alpha beta T-cell proliferations. Blood 2001;98(1):165-173

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

6. Jaffe ES, Cossman J: Immunodiagnosis of lymphoid and mononuclear phagocytic neoplasms. In Manual of Clinical Immunology. Third edition. Edited by NR Rose, H Friedman, JL Fahey. ASM Press, 1987, pp 779-790

7. Morice WG, Kimlinger T, Katzmann JA, et al: Flow cytometric assessment of TCR-V-beta expression in the evaluation of peripheral blood involvement by T-cell lymphoproliferative disorders: a comparison with conventional T-cell immunophenotyping and molecular genetic techniques. Am J Clin Pathol 2004;121(3):373-383

8. Stelzer GT, Shultz KE, Loken MR: CD45 gating for routine flow cytometric analysis of bone marrow specimens. Ann NY Acad Sci 1993;677:265-280

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.

Flow cytometric immunophenotyping of tissues is performed using the following antibodies:

Tissue Panel: CD3, CD5, CD7, CD10, CD19, CD20, CD23, CD45, 7-AAD, 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, CD45, CD138, and cytoplasmic kappa and lambda light chains

(Flow Cytometry in Clinical Diagnosis. Fourth edition. Edited by P Keren, JP McCoy Jr, J Carey. ASCP Press, Chicago, IL, 2007)

PDF Report

Supplemental

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 tissue/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

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

Additional CPTs may be added if consultative help is needed with the case, or algorithm dictates Mayo consultant involvement.

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

Test ID	Test Order Name	Order LOINC Value
LLTOF	Leukemia/Lymphoma; Tech Only Flow	In Process

Result ID	Test Result Name	Result LOINC Value
CK071	Flow Cytometry	69052-9
CK072	Final Diagnosis	22637-3
CK073	Microscopic Description	22635-7
CK074	Special Studies	30954-2