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

Test ID	Reporting Name	Available Separately	Always Performed
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
AMLF	AML, FISH	Yes	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

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

-Acute Myeloid Leukemia: Relapsed with Previous Remission Testing 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
- Acute Myeloid Leukemia: Testing Algorithm
- Acute Myeloid Leukemia: Relapsed with Previous Remission Testing Algorithm

Method Name

Immunophenotyping

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

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

For 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), order 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.**

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 fluids, or 96 hours for peripheral blood and bone marrow.

Necessary Information

The following information is required:

1. Pertinent clinical history including reason for testing 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: Whole blood

Container/Tube:

Preferred: Yellow top (ACD solution A or B)

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

Specimen Volume: 6 mL

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

Collection Instructions:

1. Send specimen in original tube. **Do not** aliquot.

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 to 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 to 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

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.

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

1. Jevremovic D, Dronca RS, Morice WG, et al: CD5+ B-cell lymphoproliferative disorders: Beyond chronic lymphocytic leukemia and mantle cell lymphoma. Leuk Res. 2010 Sep;34(9):1235-1238

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

3. Jevremovic D, Olteanu H: Flow cytometry applications in the diagnosis of T/NK-Cell lymphoproliferative disorders. Cytometry B Clin Cytom. 2019 Mar;96(2):99-115

4. Rosado FG, Morice WG, He R, Howard MT, Timm M, McPhail ED: Immunophenotypic features by multiparameter flow cytometry can help distinguish low grade B-cell lymphomas with plasmacytic differentiation from plasma cell proliferative disorders with an unrelated clonal B-cell process. Br J Haematol. 2015 May;169(3):368-376

5. Shi M, Ternus JA, Ketterling RP, et al: Immunophenotypic and laboratory features of t(11;14)(q13;q32)-positive

plasma cell neoplasms. Leuk Lymphoma. 2018;59(8):1913-1919

6. 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 Mar;121(3):373-383

7. Shi M, Jevremovic D, Otteson GE, Timm MM, Olteanu H, Horna P: Single antibody detection of T-Cell receptor alpha beta clonality by flow cytometry rapidly identifies mature T-Cell neoplasms and monotypic small CD8-positive subsets of uncertain significance. Cytometry B Clin Cytom. 2020 Jan;98(1):99-107

8. Jevremovic D, Olteanu H: Flow cytometry applications in the diagnosis of T/NK-cell lymphoproliferative disorders. Cytometry B Clin Cytom. 2019 Mar;96(2):99-115

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, TRBC1, and gamma/delta

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

-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: CD10, CD19, CD20, CD22, CD24, CD34, CD38, CD45, CD58, and 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

-Mast Cell Panel: CD2, CD25, CD69, CD117.(Keren P, McCoy JP, Carey J, eds. Flow Cytometry in Clinical Diagnosis. 4th ed. ASCP Press; 2007; Betters DM: Use of flow cytometry in clinical practice. J Adv Pract Oncol. 2015 Sep-Oct;6[5]:435-440)

PDF Report

No

Day(s) Performed

Monday through Saturday

Report Available

1 to 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 US 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

Test ID	Test Order Name	Order LOINC Value
LCMS	Leukemia/Lymphoma, Phenotype	In Process

Result ID	Test Result Name	Result LOINC Value
CK155	LCMS Result	No LOINC Needed
18255	Final Diagnosis:	34574-4
18254	Special Studies:	30954-2
18253	Microscopic Description	22635-7
18245	Accession Number	57723-9
18246	Referring Pathologist/Physician	46608-6
18247	Ref. Path Address	74221-3

Result ID	Test Result Name	Result LOINC Value
18248	Material	No LOINC Needed
18528	Specimen:	31208-2
18249	Bone Marrow Differential	No LOINC Needed
18250	Peripheral Blood:	No LOINC Needed
18251	Aspirate:	No LOINC Needed
18252	Biopsy	No LOINC Needed
18256	Comment:	48767-8
18257	Revision Description:	81317-0
18258	Signing Pathologist	19139-5
18259	Special Procedures	30954-2
18260	SP Signing Pathologist	19139-5
18261	*Previous Report Follows*	22639-9
18262	Addendum	35265-8
19191	Addendum Comment:	22638-1
18263	Addendum Pathologist:	19139-5