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
FCIMS	Flow Cytometry Interp, 9-15 Markers	No, (Bill Only)	No
FCINS	Flow Cytometry Interp,16 or greater	No, (Bill Only)	No
FCINT	Flow Cytometry Interp, 2-8 Markers	No, (Bill Only)	No
AMLF	AML, FISH	Yes	No

Additional Tests

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

Testing Algorithm

This test is designed to delay the start of leukemia/lymphoma immunophenotyping until the preliminary assessment is completed. Specimens are held in the laboratory until noon (12 p.m. Central time) 2 days after the collection date.

For testing to be cancelled, the client must call 800-533-1710. The testing process will be initiated and fully charged if no notification is received within this time period. To expedite the beginning of testing, call 800-533-1710.

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, AMLF / Acute Myeloid Leukemia (AML), FISH, Varies 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 and/or blasts and promyelocytes are CD34 and *HLA-DR*-negative.

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 and 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. 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.

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.

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 fluorescence in situ hybridization (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 these tests.

These include:

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 are performed as needed.

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

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 (BAL) and bronchial washings 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 specimen types.

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

Necessary Information

The following information is required before the specimen will be processed:

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

b. A pathology/diagnostic report including the client surgical pathology case number.

c. Clinical or morphologic suspicion

d. Specimen source

e. Date and time of collection

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

Specimen Required

Due to specimen stability, spinal fluid is not appropriate for this test.

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/Refrigerated <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/Refrigerated <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 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/Ambient <72 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, RPMI, or equivalent)

Specimen Volume: 5 mm(3) or larger biopsy

Collection Instructions:

1. Send intact specimen (do not mince).

2. Specimen cannot be fixed.

Specimen Stability Information: Ambient/Refrigerated <96 hours

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: 1 mL
Fluid: 5 mL
Tissue: 1 mm(3) or larger biopsy

Reject Due To

Gross hemolysis	Reject
Bronchoalveolar lavage (BAL) or bronchial washings Fixed, paraffin-embedded, or minced 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.

Reference Values

When performed, 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.

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 screened 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.

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, 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, and 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) and Time(s) Test Performed

Monday through Saturday

Analytic Time

2 days

Maximum Laboratory Time

4 days

Specimen Retention Time

Remaining blood/bone marrow -14 days; Remaining fluid/tissue â€“ 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)

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
HLLFH	Heme Leukemia/Lymphoma; Flow Hold V	In Process

Result ID	Test Result Name	Result LOINC Value
CK075	Final Diagnosis	34574-4
CK076	Special Studies	30954-2
CK077	Microscopic Description	22635-7
CK078	Flow Cytometry Testing	No LOINC Needed