

POLICY SECTIONS

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POLICY DESCRIPTION

Flow cytometry is a technique for live cell analysis that measures optical light scattering features to determine physical characteristics (Adan, Alizada, Kiraz, Baran, & Nalbant, 2017). This instrument is beneficial for calculating the number of cells in a biologic sample, as well as for measuring cellular properties, such as size, shape, viability, and granularity (Verbsky & Routes, 2021). Flow cytometry may also be used for diagnostic and prognostic purposes when monitoring certain diseases, and for identifying the presence of specific biomarkers.

RELATED POLICIES

Policy No.	Policy Title
M2175	Minimal Residual Disease

INDICATIONS and/or LIMITATIONS OF COVERAGE

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

- Flow cytometry immunophenotyping of cell surface markers **MEETS COVERAGE CRITERIA** for any of the following conditions:
 - Cytopenias, lymphomas, leukemia, myeloproliferative and lymphoproliferative disorders, or myelodysplastic syndrome
 - B-cell monitoring for immunosuppressive disorders
 - T-cell monitoring for HIV infection and AIDS
 - Mast cell neoplasms
 - Paroxysmal nocturnal hemoglobinuria
 - Post-operative monitoring of members who have undergone organ transplantation
 - Plasma cell disorders
 - Primary Immunodeficiencies (PIDs), and PIDs involving T, NK
 - Minimal Residual Disease (MRD)
 - Molar pregnancy
 - Primary Platelet Disorders, Non-neoplastic
 - Red Cell and White Cell Disorders, Non-neoplastic
- The following reimbursement limitations will apply for flow cytometry:
 - For flow cytometric immunophenotyping for the assessment of potential hematolymphoid neoplasia, use codes 88184-88189.
 - Code 88184 should be used for the first marker, per specimen, and is reimbursable up to a maximum of two units per date of service.
 - Code 88185 should be used for each additional marker and is reimbursable up to a maximum of 35 units, per date of service.
 - In patients with a neoplasm with an established immunophenotype, subsequent tests for that neoplasm should be limited to diagnostically relevant markers.

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- e. Codes 88187, 88188, and 88189 should not be used together in any combination. They are mutually exclusive and reimbursable as a single unit only.
- f. Codes 88187-88189 should not be used in conjunction with codes 86355, 86356, 86357, 86359, 86360, 86361, 86367.
- g. Use codes 86355, 86357, 86359, 86360, 86361, or 86367 for cell enumeration. These codes are reimbursable as single units only.

3. Coding

Bill Type Codes

012x	Hospital Inpatient (Medicare Part B only)
013x	Hospital Outpatient
014x	Hospital - Laboratory Services Provided to Non-patients
018x	Hospital - Swing Beds
021x	Skilled Nursing - Inpatient (Including Medicare Part A)
022x	Skilled Nursing - Inpatient (Medicare Part B only)
023x	Skilled Nursing - Outpatient
071x	Clinic - Rural Health
077x	Clinic - Federally Qualified Health Center (FQHC)
085x	Critical Access Hospital

Group 1 Codes:

88182	Cell marker study
88184	Flowcytometry/ tc 1 marker
88185	Flowcytometry/tc add-on
88187	Flowcytometry/read 2-8
88188	Flowcytometry/read 9-15
88189	Flowcytometry/read 16 & >

Group 2: Quantitative Codes in immunology section

Group 2 Codes:

86355	B cells total count
86356	Mononuclear cell antigen
86357	Nk cells total count
86359	T cells total count
86360	T cell absolute count/ratio
86361	T cell absolute count
86367	Stem cells total count

TABLE OF TERMINOLOGY

Term	Definition
AIDS	Acquired Immune Deficiency Syndrome
AML	Acute myeloid leukemia
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology

Term	Definition
B-ALL	B-cell precursor ALL
CAP	College of American Pathologists
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLL	Chronic lymphocytic leukemia
CMPD	Chronic myeloproliferative disorders
CMS	Centers for Medicare and Medicaid
CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
ERIC	European Research Initiative on CLL
ESCCA	European Society for Clinical Cell Analysis
FCI	Flow cytometric immunophenotyping
FCM	Flow cytometry
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridization
FISHIS	Fluorescent in situ hybridization in suspension
HIV	Human immunodeficiency virus infection
HSCT	Hematopoietic stem cell transplantation
ISTH	International Society on Thrombosis and Haemostasis
LCDs	Local coverage determinations
LDTs	Laboratory developed tests
MDS	Myelodysplastic syndromes
MFC	Multiparameter (multicolor) flow cytometry
MRD	Minimal residual disease
NCDs	National coverage determinations
NK	Natural killer
PIDs	Primary immunodeficiencies
RCUD	Refractory anemia subtype
RNA	Ribonucleic acid
RT-qPCR	Real-time quantitative polymerase chain reaction
T-ALL	T-cell acute lymphoblastic leukemia

SCIENTIFIC BACKGROUND

Flow cytometry is a laboratory technique with the capability to measure optical and fluorescence characteristics from single cells or other particles between 0.2 and 150 micrometers in size, such as microorganisms, nuclei or chromosome preparations suspended in fluid (Brown & Wittwer, 2000; Verbsky & Routes, 2021). More than 100 companies constitute the flow cytometry market, leading to an industry worth of more than \$3 billion (Robinson & Roederer, 2015).

A typical flow cytometer contains five main components: a flow cell, a laser, optical parts, detectors which amplify signals, and an electronic or computer system (Verbsky & Routes, 2021). This device measures thousands of cells instantaneously by passing them through the laser beam, and it can even sort the cells into 96- or 384-well plates, tubes, and slides based on identified cellular properties (McKinnon, 2018). Size is determined by the forward angle light scatter, and internal properties such as cellular granularity are measured by the right-angle light scatter (Brown & Wittwer, 2000; Verbsky & Routes, 2021). These fluorescent light signals are converted into electronic signals and then analyzed by a computer to generate final results (McKinnon, 2018).

Fluorescent reagents may be used to enhance a sample before administration into the flow cytometer. These reagents may include DNA binding dyes, fluorescently conjugated antibodies, viability dyes, fluorescent expression proteins, and ion indicator dyes (McKinnon, 2018). Each fluorescent dye binds to cellular components differently, leading to

distinguished outcomes when passed by the light source. A fluorochrome, or chemical that can re-emit light when excited, can assist in the detection of specific cellular properties. The use of multiple fluorochromes at once allows several characteristics to be identified instantaneously as different colors emit different wavelengths of light; common dyes include propidium iodide, phycoerythrin, and fluorescein (Brown & Wittwer, 2000).

Immunophenotyping is the most common use of flow cytometry and entails the identification of cellular markers from the immune system, such as T cell subsets and cytokines, as well as antigen-specific responses. Unfortunately, immunophenotyping faces issues in the clinical world due to a lack of standardized procedures (Finak et al., 2016). Current instruments allow for up to 28 colors to be used in immunophenotyping experiments, yet many researchers use less than this (McKinnon, 2018).

Clinical Utility and Validity

Technically, any biologic sample can be analyzed by flow cytometry. However, blood is the most common sample type, including both whole blood and peripheral blood mononuclear cells (Verbsky & Routes, 2021). Flow cytometry can be employed for prognostic and diagnostic purposes. This technique has been used to identify both primary immunodeficiencies and secondary or acquired immunodeficiencies such as HIV (Verbsky & Routes, 2021). Primary immunodeficiencies represent more than 300 known genetic disorders, and flow cytometry is a major component of the diagnosis of these disorders (Abraham & Aubert, 2016). Flow cytometry may also be used for prenatal diagnoses, hematology, transplantation, crop improvement, sperm sorting for sex preselection, post-bone marrow transplantation analyses, and during immunosuppression and chemotherapy treatments (Halder, Nath, & Jha, 2017; Verbsky & Routes, 2021).

Today, many assays have been developed for flow cytometry purposes. These assays can identify biomarkers for cancer and stem cells, DNA and RNA, reactive oxygen species, and the functional status of yeast or bacteria (Robinson & Roederer, 2015). Newer techniques have also been developed such as mass cytometry: the combination of flow cytometry and mass spectrometry (Cosma, Nolan, & Gaudilliere, 2017). Flow fluorescent in situ hybridization (FISH) is another combinatory technique which is the combination of fluorescent in situ hybridization in suspension (FISHIS) and flow cytometry using DNA or gene-specific probes.

Flow cytometry techniques have been used to identify several types of cancer. Fromm, Thomas, and Wood (2009) used flow cytometry to identify classical Hodgkin lymphoma, neoplastic Hodgkin, and Reed Sternberg cells in lymph nodes with 88.7% sensitivity and 100% specificity. Paiva, Merino, and San Miguel (2016) state that next generation multiparameter flow cytometry “should be considered mandatory in the routine evaluation of multiple myeloma patients both at diagnosis and after therapy and represents an attractive technique to integrate with high-throughput DNA and RNA-seq methods to help in understanding the mechanisms behind dissemination and chemoresistance of multiple myeloma.” Finally, Novikov et al. (2019) used flow cytometry immunophenotyping to identify malignant T-cell clones in mature peripheral T-cell lymphomas with 97% sensitivity and 91% specificity.

Wang et al. (2019) published a study on the applicability of multiparameter (multicolor) flow cytometry (MFC) for detecting MRD to predict relapse in patients with AML after allogeneic transplantation. The researchers also compared MFC to MRD status determined using real-time quantitative polymerase chain reaction (RT-qPCR) from 158 bone marrow samples from 44 different individuals. “Strong concordance was found between MFC-based and RT-qPCR-based MRD status ($\kappa = 0.868$).” Moreover, for individuals in complete remission (CR), “the positive MRD status detected using MFC was correlated with a worse prognosis [HRs (*P* values) for relapse, event-free survival, and overall survival: 4.83 (<0.001), 2.23 (0.003), and 1.79 (0.049), respectively]; the prognosis was similar to patients with an active disease before HSCT [hematopoietic stem cell transplantation] (Wang et al., 2019).”

GUIDELINES AND RECOMMENDATIONS

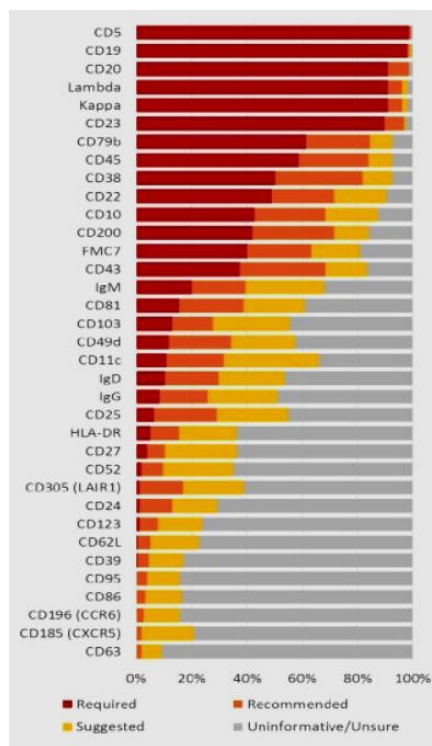
Flow cytometry is broadly used for many conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for flow cytometry.

The European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation Project

This group has published guidelines on chronic lymphocytic leukemia (CLL) in hopes to determine “35 potential flow

cytometry markers as being “required,” “recommended,” “suggested,” “uninformative,” or “not sure” for the diagnosis of CLL (Rawstron et al., 2018).” A marker is required if >75% of ERIC/ESCCA members determine that it should be required, and a marker is pushed forward for review if >50% of all members determine that it should be recommended or required. Results are shown in the following figure:

Figure 1 [taken from (Rawstron et al., 2018)]:



International/European Leukemia Net Working Group for Flow Cytometry in Myelodysplastic Syndromes

An international working party was organized to develop flow cytometry techniques in the classification of myelodysplastic syndromes (MDS). The group has stated the following guidelines:

- “In laboratories where comprehensive immunophenotyping can be performed, an MDS immunophenotyping panel... is recommended.
- In patients with cytological findings suggesting MDS of RCUD (refractory anemia subtype) or refractory anemia with ringed sideroblasts categories, aberrant flow cytometry (FCM) findings in the granulopoietic or myelomonocytic lineages may indicate multilineage dysplasia, which is of prognostic significance. Morphological findings in these cases should be thoroughly re-evaluated to avoid misclassification.
- It is important to note even small populations of myeloid progenitors with multiple immunophenotypic aberrant features (such as aberrant expression of CD7, CD56 or CD11b, see Table 1), since they indicate a higher risk of progression to AML. FCM findings in these cases should be included in the individual risk assessment (Porwit et al., 2014).”

The Clinical Cytometry Society 2006 Bethesda International Consensus

In 2006, a panel of subject matter experts convened to define the clinical indications that warrant the use of flow cytometry, as well as to identify of the reagents that should be used in the initial and secondary evaluations for those conditions (Davis et al., 2007). The output of that gathering was the 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry. The panel indicated that flow cytometry is useful for the evaluation of cytopenias, elevated leukocyte count, observation of atypical cells or blasts and evaluation of body fluids, plasmacytosis or monoclonal gammopathy, organomegaly and tissue masses, and certain patient monitoring indications.

The Bethesda recommendations indicate that flow cytometry is not indicated for mature neutrophilia, polyclonal hypergammaglobulinemia, polycythemia, thrombocytosis, and basophilia because “they are usually not associated with hematolymphoid malignancy or associated with hematolymphoid neoplasms that are not detectable by” flow cytometry.

The Bethesda recommendations also indicate that selection of reagents for the initial evaluation panel should be based on specimen type (peripheral blood, bone marrow, tissue, etc.), clinical information and cell morphology studies. They identify initial panels for specific indications that range from a total of 4 reagents to a maximum of 12 reagents.

For secondary evaluation, where the initial evaluation is not conclusive or informative, the Bethesda recommendations again identify groups of reagents that should be used, based on indication. The secondary panels ranged from 5 to 23 reagents.

Specific recommendations for the initial evaluation were:

- B cells: CD5, CD10, CD19, CD20, CD45, Kappa, Lambda
- T cells and NK cells: CD2, CD3, CD4, CD5, CD7, CD8, CD45, CD56
- Myelomonocytic cells: CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, CD117, HLA-DR
- Myelomonocytic cells (limited): CD13, CD33, CD34, CD45
- Plasma cells CD19, CD38, CD45, CD56

For secondary evaluation, the Bethesda recommendations were:

- B cells: CD9, CD11c, CD15, CD22, cCD22, CD23, CD25, CD13, CD33, CD34, CD38, CD43, CD58, cCD79a, CD79b, CD103, FMC7, Bcl-2, cKappa, cLambda, TdT, Zap-70, clgM
- T cells and natural killer (NK) cells: CD1a, cCD3, CD10, CD16, CD25, CD26, CD30, CD34, CD45RA, CD45RO, CD57, ab-TCR, gd-TCR, cTIA-1, T-beta chain isoforms, TdT
- Myelomonocytic cells: CD2, CD4, CD25, CD36, CD38, CD41, CD61, cCD61, CD64, CD71, cMPO, CD123, CD163, CD235a
- Plasma cells: CD10, CD117, CD138, cKappa, cLambda

The American Society of Clinical Oncology Tumor Markers Expert Panel (ASCO)

In 2006, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of gastrointestinal cancers. These recommendations state that “Neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer (Locker et al., 2006).”

In 2007, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer (Harris et al., 2007); the authors noted that “DNA/ploidy by flow cytometry demonstrated insufficient evidence to support routine use in clinical practice.”

College of American Pathologists and the American Society of Hematology

In 2016, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) published a joint guideline to outline their recommendations for the initial diagnostic workup of acute leukemia. Among their 27 recommendations, three statements (each rated “Strong Recommendation”) explicitly address the leveraging of flow cytometry in said process:

“5. In addition to morphologic assessment (blood and bone marrow), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (i.e., karyotype), appropriate molecular genetic and/or fluorescent in situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI). The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-cell acute lymphoblastic leukemia (T-ALL) (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. FISH and/or molecular genetic testing does not, however, replace conventional cytogenetic analysis.

Note — If sufficient bone marrow aspirate or peripheral blood material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second bone marrow core biopsy can be obtained and submitted, unfixed in tissue culture media, for disaggregation for genetic studies and flow cytometry.”

“10. For patients with suspected or confirmed acute leukemia, the pathologist may use flow cytometry for the evaluation of CSF.”

“12. For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease.”

A final recommendation (also a “Strong Recommendation”) mentioning flow cytometry referred to the use of its data, such that

“24. If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available.”

International Society on Thrombosis and Haemostasis (ISTH)

The International Society on Thrombosis and Haemostasis SSC Subcommittee outlined several recommendations for which flow cytometric analysis of inherited and acquired platelet disorders should occur. Those clinical settings in which it believed would be helpful, guided by expert consensus, are reported below:

“Diagnosis of inherited or acquired deficiencies of platelet surface glycoproteins (BSS, GT, inherited or immune-mediated GPVI defects)

Diagnosis of platelet alpha granule secretion defects (such as gray platelet syndrome)

Diagnosis of defects in specific platelet activation (signaling) pathways (such as RASGRP2, P2Y12, or TXA2R disorders)

Diagnosis of GFI1B macrothrombocytopenia associated to platelet expression of CD34

Diagnosis of disorders of platelet procoagulant activity (such as Scott syndrome and Stormorken syndrome)

Assessment of increased platelet activation in prothrombotic syndromes (diabetes, anti-phospholipid syndrome or secondary to drug induced, non-immune platelet activation)

Monitoring, if applicable, pharmacodynamic effect of P2Y12 antagonists (ticlopidine, clopidogrel, prasugrel, ticagrelor, cangrelor) with specifically designed test such as VASP P2Y12

Determination of the fraction of immature platelets” (Frelinger et al., 2021).

APPLICABLE STATE AND FEDERAL REGULATIONS

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member (e.g., Local Coverage Determinations [LCDs]) or National Coverage Determinations [NCDs] for Medicare and/or state coverage for Medicaid), then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the [Medicare search website](#). For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

APPLICABLE CPT / HCPCS PROCEDURE CODES

CPT	Code Description
86355	B cells, total count
86356	Mononuclear cell antigen, quantitative (e.g., flow cytometry), not otherwise specified, each antigen
86357	Natural killer (NK) cells, total count
86359	T cells; total count
86360	T cells; absolute CD4 and CD8 count, including ratio
86361	T cells; absolute CD4 count
86367	Stem cells (e.g., CD34), total count
88182	Flow cytometry, cell cycle or DNA analysis
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
88187	Flow cytometry, interpretation; 2 to 8 markers
88188	Flow cytometry, interpretation; 9 to 15 markers
88189	Flow cytometry, interpretation; 16 or more markers

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 Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Coding Notes:

- For flow cytometric immunophenotyping for the assessment of potential hematolymphoid neoplasia, use 88184-88189.
- Do not report 88187-88189 for interpretation of 86355, 86356, 86357, 86359, 86360, 86361, and 86367.

Approval History

Type	Date	Action
Effective Date	7/1/2022	New Policy
Revision Date		

EVIDENCE-BASED SCIENTIFIC REFERENCES

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APPENDIX

Reserved for State specific information. Information includes, but is not limited to, State contract language, Medicaid criteria and other mandated criteria.