Overview

Useful For
Evaluating patients with clinical features of IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance) and other primary immunodeficiencies, autoimmune diseases, allergy and asthma, and graft-vs-host disease post-hematopoietic stem cell transplantation.

Method Name
FlowCytometry

NY State Available
No

Specimen

Specimen Type
Whole Blood EDTA

Shipping Instructions
Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday. Draw and package specimen as close to shipping time as possible.

It is recommended that specimens arrive within 24 hours of draw.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information
Ordering physician's name and phone number are required.

Specimen Required
For serial monitoring, we recommend that specimen draws be performed at the same time of day.

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions: Send specimen in original tube. Do not aliquot.

Specimen Minimum Volume
1 mL

Reject Due To

<table>
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<tr>
<th>Condition</th>
<th>Action</th>
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<tr>
<td>Gross hemolysis</td>
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<tr>
<td>Gross lipemia</td>
<td>Reject</td>
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Specimen Stability Information
Test Definition: TREGS
T Cell Subsets, Regulatory (Tregs)

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tr>
<td>Whole Blood EDTA</td>
<td>Ambient</td>
<td>72 hours</td>
<td>PURPLE OR PINK TOP/EDTA</td>
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Clinical and Interpretive

Clinical Information

Regulatory T cells (Tregs) are a population of CD4+ T cells with a unique role in the immune response. Tregs are crucial in suppressing aberrant pathological immune responses in autoimmune diseases, transplantation, and graft-vs-host disease after allogeneic hematopoietic stem cell transplantation. Tregs are activated through the specific T-cell receptor, but their effector function is nonspecific and they regulate the local inflammatory response through cell-to-cell contact and cytokine secretion. Tregs secrete interleukin (IL)-9 (IL-9), IL-10, and transforming growth factor-beta 1 (TGF-beta 1), which aid in the mediation of immunosuppressive activity.

Chief characteristics of the Treg population are surface expression of the CD25 protein (IL-2Ra) and the intracellular presence of the transcription factor Foxp3. The IL-7 receptor (CD127) is downregulated on Foxp3+CD4+CD25+ T cells and provides an excellent alternative cell-surface marker to Foxp3 for detecting natural Tregs (CD4+CD25+CD127lo). Natural Tregs account for 5% to 10% of the total CD4 T-cell population and are derived from thymic precursors. Since CD25 is also expressed on activated T cells, the concomitant use of CD127 permits the differentiation of Tregs from activated T cells. Natural Tregs express the memory marker CD45RO and have limited ability to proliferate. However, within the CD4+CD25+Treg population, there is a subset of Tregs that express the CD45 isoform generally associated with naive T cells (CD45RA), and this subset has been called natural naive (Nn) Tregs. Nn Tregs are most prominent in young adults and decrease with age along with the rest of the naive CD4 T-cell population. Like other naive T cells, Nn Tregs have high proliferative capacity, as well as the suppressor activity of other Treg subsets. Present evidence suggests that Nn Tregs also have a thymic ancestry and are the precursors of the natural Tregs (that are of the memory, antigen-experienced phenotype) and appear to be composed of T cells with self-reactive T-cell receptors.

Other subsets of Tregs include the Th3 cells, which secrete high levels of TGF-beta 1 and can be induced by oral administration of antigen, and regulatory T class 1 (Tr1) cells, which secrete interferon-gamma and IL-10. These Treg subsets are most likely induced in the periphery and are responsible for peripheral tolerance to self antigens. The suppressive activity of Th3 and Tr1 cells are related to the cytokines they produce, TGF-beta 1 and IL-10, respectively.

The absence of Tregs as a result of mutations in the FOXP3 gene cause a primary immunodeficiency called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance). Patients with IPEX have a complex manifestation of symptoms including severe watery diarrhea due to significant villous atrophy and lymphocytic infiltration of bowel mucosa, early-onset autoimmune endocrinopathies involving the pancreas or thyroid, and a dermatitic (eczematous) rash. In addition, there are other autoimmune manifestations including autoimmune cytopenias and autoimmune hepatitis. Renal disease is quite common in these patients. Finally, these patients also have a significant predisposition to infections including sepsis, pneumonia, meningitis, and osteomyelitis. Decreased Foxp3+CD4+CD25+Tregs have been reported in 1 patient with a STAT5b mutation.

There is an expansion of Nn Tregs in patients with monoclonal gammopathy of undetermined significance and multiple myeloma, likely as a response to the process of malignant transformation. Expansion of Tregs has also been reported in other neoplasias including B-cell chronic lymphocytic leukemia, Hodgkin disease, and solid tumors.
The absolute counts of lymphocyte subsets are known to be influenced by a variety of biological factors, including hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell count throughout the day, while CD8 T cells and CD19+ B cells increase between 8:30 am and noon, with no change between noon and afternoon. Natural killer cell counts, on the other hand, are constant throughout the day.(9) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(10-12) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(10) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening,(13) and during summer compared to winter.(14) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

**Reference Values**

The appropriate age-related reference values will be provided on the report.

**Interpretation**

The lack of regulatory T (Treg) cells is associated with mutations in the FOXP3 gene. Low Tregs are also seen in the context of STAT5b mutations. Reduced Nn Tregs and natural Tregs are likely to predispose to autoimmunity, while reductions in Th3/Tr1 cells may impair oral and peripheral tolerance, also facilitating the development of autoimmunity.

The presence of expanded naive Tregs may indicate a process of malignant transformation, if other clinical features of malignant disease are present.

Increased Tregs in donor stem cell allografts have been associated with a reduced incidence of graft-versus-host disease (ie, mediating a protective effect) after allogeneic stem cell transplantation.

**Cautions**

This panel provides only quantitative information regarding the various regulatory T cell (Treg) subsets; it does not provide information on the functional aspect of these populations.

Results should be correlated with clinical presentation.

Molecular testing is required to confirm a diagnosis of IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance). Call 800-533-1710 for assistance in ordering molecular testing.

Treg cells may be reduced in a variety of clinical contexts such as in autoimmune diseases or allograft rejection.

Timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets. See data under Clinical Information.

**Clinical Reference**


**Performance**

**Method Description**

EDTA anticoagulated blood is incubated with antibodies to various T-cell markers (i.e., CD4, CD127, CD45RO, CD45RA, and CD25). After RBC lysis, the sample is washed to remove any unbound antibodies prior to analysis on a BD FACSCantoA (6-color) flow cytometry instrument. The assay uses 2 antibody tubes for data acquisition, but analysis is performed as a single panel. Each Treg subset is expressed as a percentage of total CD4+ T cells. BD Tru Count tubes are used to calculate the absolute count of cells per mcL for each subset. The regulatory T-cell panel is linked to the TCD4 test (TCD4/84348 CD4 Count for Immune Monitoring, Blood) within the experiment and, therefore, the CD3, CD4, and CD8 T-cell reference ranges are provided within the TCD4 assay. The regulatory T cell results are interpreted using a reference range derived from data of normal healthy adult and pediatric donors. Isotype controls are used in each assay to measure background fluorescence of the samples. A normal, healthy control is also included in each experiment to ensure the optimal performance of the assay. (Unpublished Mayo information)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**
Monday through Friday

Do not send specimen after Thursday. Specimen must be received by 10 a.m. on Friday.

Analytic Time
3 days

Maximum Laboratory Time
4 days

Specimen Retention Time
4 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
86359-T cells, total count
86361-Absolute CD4 count

LOINC® Information

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