Test Definition: TREGS

T-Cell Subsets, Regulatory (Tregs), Blood

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

Flow Cytometry

NY State Available

No

Specimen

Specimen Type

Whole Blood EDTA

Shipping Instructions

Testing performed Monday through Friday. Specimens not received by 4 p.m. Central time on Fridays may be canceled

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

Collect and package specimen as close to shipping time as possible. It is recommended that specimens arrive within 24 hours of collection.

Necessary Information

The ordering healthcare professional's name and phone number are required.

Specimen Required

Container/Tube: Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**
Additional Information: For serial monitoring, it is recommended that specimens are collected at the same time of day.

Specimen Minimum Volume

1 mL

Reject Due To

Gross	Reject

hemolysis	
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Ambient	72 hours	PURPLE OR PINK TOP/EDTA

Clinical & 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.(1) 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.(2) Tregs secrete interleukin (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 alternative cell-surface marker to FOXP3 for detecting natural Tregs (CD4+CD25+CD127lo).(2)

Natural Tregs account for 5% to 10% of the total CD4 T-cell population and are derived from thymic precursors.(3) Since CD25 is also expressed on activated T cells, the concomitant use of CD127 permits the differentiation of Tregs from activated T cells.(4) 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.(5) Like other naive T cells, Nn Tregs have high proliferative capacity, as well as the suppressor activity of other Treg subsets. 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.(5)

Other subsets of Tregs include the T-helper 3 (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.(5) 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 variants in the *FOXP3* gene causes a primary immunodeficiency called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance).(6) 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. Kidney disease is quite common in these patients. Finally, these patients also have a significant predisposition to infections including sepsis, pneumonia, meningitis, and osteomyelitis.(6) Decreased

FOXP3+CD4+CD25+Tregs have been reported in other inborn errors of immunity.(7)

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.(8) 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 negatively correlate 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 cells (Tregs) is associated with variants in the *FOXP3* gene. Low Tregs are also seen in the context of other inborn errors of immunity. 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). For assistance in ordering molecular testing, call 800-533-1710.

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 Clinical Information.

Clinical Reference

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Performance

Method Description

EDTA-anticoagulated blood is incubated with antibodies to various T-cell markers (ie, CD4, CD127, CD45RO, CD45RA, and CD25). After red blood cell lysis, the sample is washed to remove any unbound antibodies prior to analysis. 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. The regulatory T-cell panel is linked to the TCD4 test (TCD4 / 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) Performed
Monday through Friday

Report Available
3 to 4 days

Specimen Retention Time
4 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & 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 account representative. 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
86359
86361

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
TREGS	T Cell Subsets, Regulatory (Tregs)	90413-6

Result ID	Test Result Name	Result LOINC® Value

29149	CD4+CD25+CD127loCD45RA+ Naive Tregs	89315-6
29148	CD4+CD25+CD127loCD45RO+ (Nat Tregs)	89316-4
29147	Activated CD4+ T cells (4+CD25+)	26982-9
29145	% N. Naive Tregs	89319-8
29144	% Natural Tregs	89320-6
29143	% Activated CD4+ T cells (4+CD25+)	13332-2
29146	% CD4+CD25-CD127+ (Tr1/Th3)	89318-0
29150	CD4+CD25-CD127+ (Tr1/Th3)	89317-2
29177	Interpretation	69052-9
609282	CD4 (T Cells)	24467-3