Overview

Useful For

Determining the presence of naive, memory, and activated T cells in various clinical contexts including autoimmune diseases, immunodeficiency states, T-cell recovery posthematopoietic stem cell transplant, DiGeorge syndrome, and as a measure for T-cell immune competence

Naive T-cells results can be used as a surrogate marker for thymic-derived T-cell reconstitution, when used in conjunction with assessment of T-cell receptor excision circles (TREC / T-Cell Receptor Excision Circles [TREC] Analysis for Immune Reconstitution)

Assessing a patient's relative risk for infections

Evaluation of patients with cellular or combined primary immunodeficiencies

Evaluation of T-cell reconstitution after hematopoietic stem cell transplant, chemotherapy, biological therapy, immunosuppression or immunomodulator therapy

Evaluation of patients with autoimmune diseases

Evaluation of HIV-positive patients for naive and memory subsets

Evaluation of T-cell immune competence (presence of memory and activated T cells) in patients with recurrent infections

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

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

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

Clinical and Interpretive

Clinical Information

T cells, after completing development and initial differentiation in the thymus, enter the periphery as naive (n) T cells. Naive T cells undergo further differentiation into effector and memory T cells in the peripheral lymphoid organs after recognizing specific antigenic peptides in the context of major histocompatibility (MHC) molecules, through the antigen-specific T-cell receptor. In addition to the cognate signal of the peptide-MHC complex interaction (the term cognate refers to 2 biological molecules that normally interact), T cells require additional costimulatory signals to complete T-cell activation. Naive T cells circulate continuously through the lymph nodes and, on recognition of specific antigen, undergo activation. Due to their antigen-inexperienced state, naive T cells require activation by more potent antigen-presenting cells, such as dendritic cells.

Naive T cells can survive in circulation for prolonged periods of time and are very important in contributing to T-cell repertoire diversity. They proliferate in response to interleukin-2, as a consequence of their response to antigen through recognition of peptide-MHC costimulation. These expanded antigen-specific T cells undergo further differentiation into effector cells. The differentiation of naive CD8 T cells into cytotoxic effectors capable of killing target T cells loaded with endogenous peptides on MHC class I molecules may require additional costimulatory signals from CD4 T cells. Naive CD4 T cells also differentiate into different effector subsets such as Th1, Th2, and Th17, which produce specific cytokines.(1)

T cells can be subdivided into naive and memory subsets based on the expression of cell-surface markers, such as CD45RA and CD45RO, among others. It was initially thought that the presence of cell-surface CD45RA indicated the naive subset, while the presence of CD45RO indicated memory subsets. But, it has now been shown that multiple, rather than single, markers are required to distinguish these subsets.(2) Lanzavecchia and Sallusto proposed a model where naive T cells expressing CD45RA and CCR7 lose CD45RA expression on recognition of antigen.(3) The surface markers for identifying naive T-cell subsets include CD45RA, CD62L (L-selectin), and CD27.(4,5)

Memory T cells are antigen-experienced cells that are present in greater numbers than antigen-specific precursors, and can respond more efficiently and rapidly to specific antigen. Memory T cells can maintain their populations independent of antigen by homeostatic proliferation in response to cytokines. While there are subcategories of memory T cells based on effector function and cell surface and cytolytic molecule expression, the 2 main categories of memory T cells are central memory T cells (Tcm) and effector memory T cells (Tem).(1,6)

Tcm express the CD45RO molecule along with CD62L (L-selectin) and CCR7, and are present mainly in lymphoid tissue.(6,7) They are able to respond to antigen through rapid proliferation and expansion and differentiation into Tem. By themselves, Tcm are not directly effective in effector cytolytic function.

Unlike Tcm, Tem express only CD45RO (not CD62L and CCR7).(6) As the name suggests, Tem have remarkable effector function, though they do not proliferate well. Tem are present throughout the circulation in peripheral tissues providing immune surveillance.

Memory T cells are particularly important for maintenance of immune competence since they are associated with a rapid and effective response to pathogens. Therefore, depletion of this compartment has more immediate significance than the depletion of naive T cells.

Activation of human T cells is critical for the optimal and appropriate performance of T-cell functions within the adaptive immune response. Activated naive T cells undergo proliferation, as well as subsequent differentiation into effector T cells, and are capable of producing cytokines that can modulate the immune response in a variety of ways.(8) There are several markers associated with T-cell activation, but those most commonly used include CD25 (IL-25R)(8) and MHC class II.(9) Additionally, the expression of the costimulatory molecule CD28 augments the T-cell activation response.(10)

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.(11) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(12-14) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(11) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening(15), and during summer compared to winter.(16) 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

Absence or reduction of naive T cells with or without T-cell lymphopenia indicates absent or impaired T-cell reconstitution or thymic output. Reduction in activated T cells can also indicate a reduced T-cell immune competent state.

Increases in naive T cells with corresponding decreases in the memory T-cell compartment indicates a failure of further differentiation and effector function or selective loss of memory T cells and an increased risk for infection.

Cautions

This assay provides quantitative information on various T-cell subsets in blood; it does not provide any information on the antigen-specific or otherwise functional state of the T cells. To assess the overall functional state of T cells, LPMGF / Lymphocyte Proliferation to Mitogens, Blood and LPAGF / Lymphocyte Proliferation to Antigens, Blood (using *Candida* and tetanus antigens) are appropriate. To assess cytomegalovirus (CMV)-specific immune

competence, order CMVC8 / Cytomegalovirus (CMV) CD8 T-Cell Immune Competence, Quantitative Assessment by Flow Cytometry.

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

Clinical Reference

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12. Dimitrov S, Benedict C, Heutling D, et al: Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood 2009;113(21):5134-5143

13. Dimitrov S, Lange T, Nohroudi K, Born J: Number and function of circulating antigen presenting cells regulated by sleep. Sleep 2007;30:401-411

14. Kronfol Z, Nair M, Zhang Q, et al: Circadian immune measures in healthy volunteers: relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. Psychosom Med 1997;59:42-50

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1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J AIDS 1990;3:144-151

16. Paglieroni TG, Holland PV: Circannual variation in lymphocyte subsets, revisited. Transfusion 1994;34:512-516

Performance

Method Description

This flow cytometric assay quantitates the following CD4 and CD8 T-cell subsets: naive (global and CD62L+), memory (global, central, and effector memory), and activated (CD4+25+ and MHC class II-positive) T cells. EDTA anticoagulated blood is incubated with antibodies to various T-cell markers (ie, CD3, CD4, CD8, CD45RO, CD45RA, HLADR, CD27, CD62L, CD25, and CD28). After RBC lysis, the sample is washed to remove any unbound antibodies prior to analysis on a BD FACS Cantoinstrument. The assay uses 4 antibody tubes for data acquisition and analysis is performed as 3 panels. Each T-cell subset is expressed as a percentage of total CD4+ or CD8 T cells. Only the CD3 T cells are expressed as a percentage of total lymphocytes. The absolute counts for the T-cell subsets are derived from flow cytometry analysis of whole blood using monoclonal antibodies to identify CD45, CD3, CD4, and CD8. CD14 is used to exclude monocytes, thereby improving accuracy and enhancing the purity of the lymphocyte population. The assay is a 5-color, lyse-no wash procedure and the absolute counts are calculated from internal bead standards. The T-cell subsets 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 results for the other T-cell subsets 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

86356 x 7

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
TCP	T Cell Phenotyping, Advanced	In Process

Result ID	Test Result Name	Result LOINC Value
29151	%CD4+CD45RA+ naive T cells	89360-2
29152	%CD4+CD62L+CD27+ naive T cells	89340-4
29153	%CD8+CD45RA+ naive T cells	In Process
29154	%CD8+CD62L+CD27+naive T cells	89339-6
29155	%CD4+CD45RO+ memory T cells	89362-8
29156	%CD4+CD62L+CD27+CD45RO+ (Tcm)	89338-8
29157	%CD4+CD62L-CD27-CD45RO+ (Tem)	89337-0
29158	%CD8+CD45RO+ memory T cells	89336-2
29159	%CD8+CD62L+CD27+CD45RO+ (Tcm)	89335-4
29160	%CD8+CD62L-CD27-CD45RO+ (Tem)	89334-7
29161	%Activated CD4 T cells (4+CD25+)	89431-1
29162	%CD4+HLA DR+CD28+ T cells	89333-9
29163	%CD8+HLA DR+CD28+ T cells	89332-1
29164	CD4+CD45RA+ naive T cells	In Process
29165	CD4+CD62L+CD27+ naive T cells	89331-3
29166	CD8+CD45RA+ naive T cells	82743-6
29167	CD8+CD62L+CD27+naive T cells	89330-5
29168	CD4+CD45RO+ memory T cells	In Process
29169	CD4+CD62L+CD27+CD45RO+ (Tcm)	89329-7
29170	CD4+CD62L-CD27-CD45RO+ (Tem)	89328-9
29171	CD8+CD45RO+ memory T cells	85790-4
29172	CD8+CD62L+CD27+CD45RO+ (Tcm)	In Process

Result ID	Test Result Name	Result LOINC Value
29173	CD8+CD62L-CD27- CD45RO+ (Tem)	89327-1
29174	Activated CD4 T cells (4+CD25+)	26982-9
29175	CD4+HLA DR+CD28+ T cells	89326-3
29176	CD8+HLA DR+CD28+ T cells	89325-5
29178	Interpretation	69052-9