# Test Definition: TCP

T-Cell Subsets, Naive, Memory, and Activated, Blood

## 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 post-hematopoietic 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 (TRECS / T-Cell Receptor Excision Circles Analysis, Blood)

Assessing a patient's relative risk for infections

Evaluating patients with cellular or combined primary immunodeficiencies

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

Evaluating patients with autoimmune diseases

Evaluating patients who are HIV-positive for naive and memory subsets

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

### Method Name

Flow Cytometry

### NY State Available

No

## Specimen

### Specimen Type

Whole Blood EDTA

### Ordering Guidance

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, order either LPMGF / Lymphocyte Proliferation to Mitogens, Blood or LPAGF / Lymphocyte Proliferation to Antigens, Blood (using *Candida* and tetanus antigens).

To assess cytomegalovirus (CMV)-specific immune competence, order CMVC8 / Cytomegalovirus (CMV) CD8 T-Cell Immune Competence, Quantitative Assessment by Flow Cytometry, Blood.

## 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

**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 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 & Interpretive

### Clinical Information

T cells, after completing development and initial differentiation in the thymus, enter the periphery as naive 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. 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 a 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 can respond to antigens 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 a.m. 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 the timing of blood collection are 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

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

### Clinical Reference

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through T-cell specific occupation of class II transactivator promoter III. J Immunol. 2002;168(2):763-770
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## 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 major histocompatibility [MHC] class II-positive) T cells. EDTA-anticoagulated blood is incubated with antibodies to various T-cell markers (ie, CD3, CD4, CD8, CD45RO, CD45RA, HLA-DR, CD27, CD62L, CD25, and CD28). After red blood cell lysis, the sample is washed to remove any unbound antibodies. 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. 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) 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
86356 x 7

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| TCP | T Cell Phenotyping, Advanced | 96493-2 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 29178 | Interpretation | 69052-9 |
| 29174 | Activated CD4 T cells (4+CD25+) | 26982-9 |
| 29165 | CD4+CD62L+CD27+ naive T cells | 89331-3 |
| 29169 | CD4+CD62L+CD27+CD45RO+ (Tcm) | 89329-7 |
| 29170 | CD4+CD62L-CD27-CD45RO+ (Tem) | 89328-9 |
| 29167 | CD8+CD62L+CD27+naive T cells | 89330-5 |
| 29172 | CD8+CD62L+CD27+CD45RO+ (Tcm) | 96492-4 |
| 29173 | CD8+CD62L-CD27- CD45RO+ (Tem) | 89327-1 |
| 29175 | CD4+HLA DR+CD28+ T cells | 89326-3 |
| 29176 | CD8+HLA DR+CD28+ T cells | 89325-5 |
| 29161 | %Activated CD4 T cells (4+CD25+) | 89431-1 |
| 29152 | %CD4+CD62L+CD27+ naive T cells | 89340-4 |
| 29156 | %CD4+CD62L+CD27+CD45RO+ (Tcm) | 89338-8 |

| | | |
|---|---|---|
| 29157 | %CD4+CD62L-CD27-CD45RO+ (Tem) | 89337-0 |
| 29154 | %CD8+CD62L+CD27+naive T cells | 89339-6 |
| 29159 | %CD8+CD62L+CD27+CD45RO+ (Tcm) | 89335-4 |
| 29160 | %CD8+CD62L-CD27-CD45RO+ (Tem) | 89334-7 |
| 29162 | %CD4+HLA DR+CD28+ T cells | 89333-9 |
| 29163 | %CD8+HLA DR+CD28+ T cells | 89332-1 |
| 29151 | %CD4+CD45RA+ naive T cells | 89360-2 |
| 29153 | %CD8+CD45RA+ naive T cells | 82744-4 |
| 29155 | %CD4+CD45RO+ memory T cells | 89362-8 |
| 29158 | %CD8+CD45RO+ memory T cells | 89336-2 |
| 29164 | CD4+CD45RA+ naive T cells | 26759-1 |
| 29166 | CD8+CD45RA+ naive T cells | 82743-6 |
| 29168 | CD4+CD45RO+ memory T cells | 85792-0 |
| 29171 | CD8+CD45RO+ memory T cells | 85790-4 |
| 609282 | CD4 (T Cells) | 24467-3 |
| 609283 | CD8 (T Cells) | 14135-8 |