MAYO CLINIC LABORATORIES

# Test Definition: TRECS

T-Cell Receptor Excision Circles Analysis, Blood

## Overview

### Useful For

Measuring T-cell output or reconstitution (thymopoiesis) following hematopoietic cell transplantation or highly active antiretroviral therapy

Evaluating thymic function in patients with cellular or combined inborn errors of immunity (formerly primary immunodeficiencies), or receiving immunotherapy or cancer vaccines

Assessing T-cell recovery following thymus transplants for DiGeorge syndrome

### Special Instructions

- TREC Assay Patient Information

### Method Name

Real-Time Quantitative Polymerase Chain Reaction (PCR)

### NY State Available

Yes

## Specimen

### Specimen Type

Whole Blood EDTA

### Additional Testing Requirements

This assay is useful for evaluating thymic output, and for longitudinal assessment of thymic function.

For comprehensive assessment of thymic function in pediatric patients and/or individuals who have received hematopoietic stem cell transplantation, order this test together with CD4RT / CD4 T-Cell Recent Thymic Emigrants, Blood.

### Shipping Instructions

**Specimens must be received in the laboratory on weekdays and by 4 p.m. on Friday.** Collect and package specimen as close to shipping time as possible.

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

Samples arriving over the weekend or on observed holidays may be canceled.

### Necessary Information

**Ordering physician's name and phone number are required.**

**TREC Assay Patient Information** (T589) **is required.** Testing will proceed without the form; however, results will be held until the information is received.

## Specimen Required

For serial monitoring, it is recommended to perform specimen collection at the same time of day, if possible.
**Supplies:** Ambient Shipping Box-Critical Specimens Only (T668)
**Container/Tube:** Lavender top (EDTA)
**Specimen Volume:**
Adults: 10 mL
Pediatrics
-Preferred volume for >1 year: 5 mL
-Preferred volume for < or =1 year old: 3 mL
**Collection Instructions:**
**1. Do not collect specimen using a butterfly needle.**
2. Send whole blood specimen in original tube. **Do not aliquot.**

## Forms

**TREC Assay Patient Information** (T589) **is required**

## Specimen Minimum Volume

Adults: 10 mL
Pediatrics: 1 mL

## Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| Gross lipemia | Reject |

## Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Whole Blood EDTA | Ambient | 48 hours | PURPLE OR PINK TOP/EDTA |

## Clinical & Interpretive

### Clinical Information

T-cell generation is a critical feature of the adaptive immune response and has 2 main components: thymic output of new T cells and peripheral homeostatic expansion of preexisting T cells. It has been shown that although thymic function declines with age, a reasonable output is still maintained into late adult life.(1) In many clinical situations, thymic output is crucial to the maintenance and competence of the T-cell effector immune response.

Thymic output of new T cells can be determined by T-cell receptor excision circles (TREC) analysis. TREC are extrachromosomal DNA byproducts of T-cell receptor (TCR) rearrangement, which are nonreplicative. TREC are produced only in T cells of thymic origin and each cell is thought to contain a single copy of the TREC measured in this test. Hence, TREC analysis provides a specific assessment of T-cell recovery (eg, after hematopoietic stem cell transplantation) or numerical T-cell competence. There are several TREC generated during the process of TCR rearrangement and the TCR delta deletion TREC (deltaREC psi-J-alpha signal joint TREC) has been shown to be the most accurate TREC for measuring thymic output.(2) This assay measures this specific TREC using quantitative, real-time polymerase chain reaction.

Clinical use of TREC in HIV and Antiretroviral Therapy:
HIV infection leads to a decrease in thymic function. Adult patients treated with highly active antiretroviral therapy (HAART) show a rapid and sustained increase in thymic output.(1)

Clinical use of TREC in Hematopoietic Stem Cell Transplantation and Inborn Errors of Immunity (formerly Primary Immunodeficiencies)(3):
There is a period of immunodeficiency following hematopoietic stem cell transplantation (HSCT) that varies depending on the nature and type of stem cell graft used and the conditioning regimen, among other factors. This secondary immunodeficiency also includes defects in thymopoiesis.(4-6) It has been shown that numerical T-cell recovery is usually achieved by day 100 post-transplant, although there is an inversion of the CD4:CD8 ratio that can persist for up to a year.(5) Also, recovery of T-cell function and diversity can take up to 12 months, although this can be more rapid in pediatric patients. However, recovery of T-cell function is only possible when there is numerical reconstitution of T cells. T cells, along with the other components of adaptive immunity, are key players in the successful response to vaccination post-HSCT.(7)

In patients who have received HSCT for severe combined immunodeficiency, T-cell recovery early after transplant is crucial to long-term T-cell reconstitution.(8) Patients who demonstrated impaired reconstitution were shown to have poor early grafting, as opposed to immune failure caused by accelerated loss of thymic output or long-term graft failure. In this study, the numbers of TREC early after HSCT were most predictive for long-term reconstitution. The data suggests that frequent monitoring of T-cell immunity and TREC numbers after HSCT can help identify patients who will fail to reconstitute properly, which would allow institution of additional therapies in a timely manner.(8) It would be reasonable to extrapolate such a conclusion to other diseases that are also treated by HSCT.

TREC Copies and Thymic Output in Adults:
Since the adult thymus involutes after puberty and is progressively replaced by fat with age, thymus-dependent T-cell recovery has been assumed to be severely limited in adults. However, with TREC analysis it has been shown that the change in thymic function in adults is a quantitative phenomenon rather than a qualitative one and thymic output is not totally eliminated.(1,9,10) Thus, after HSCT or HAART, the remaining thymic tissue can be mobilized in adults to replenish depleted immune systems with a potentially broader repertoire of naive T cells. Douek et al have shown that there is a significant contribution by the thymus to immune reconstitution after myeloablative chemotherapy and HSCT in adults.(9) In fact, this data show that there is both a marked increase in the TREC numbers and a significant negative correlation of TREC copies with age post-transplant.

In addition to the specific clinical situations elucidated above, TREC analysis can be helpful in identifying patients with primary immunodeficiencies and assessing their numerical T-cell immune competence. It can also be used as a measure of immune competence in patients receiving immunotherapy or cancer vaccines, where maintenance of T-cell output is

integral to the immune response against cancer.

The absolute counts of lymphocyte subsets are influenced by a variety of biological factors, including genetic background, hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell counts 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 cells are 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.(12) 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 consistency in 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

T-cell receptor excision circles (TREC) generally show an inverse correlation with age, although there can be substantial variations in TREC copies relative to T-cell count within a given age group.

Following hematopoietic stem cell transplantation (HSCT), highly active antiretroviral therapy (HAART), thymic transplants, etc, TREC typically increases from absent or very low levels (below age-matched reference range) to baseline levels or exceeds baseline levels, showing evidence of thymic rebound, which is consistent with recovery of thymic output and T-cell reconstitution.

When a patient is being monitored for thymic recovery post-transplant, it is recommended that a pre-transplant (prior to myeloablative or non-myeloablative conditioning) or a pretreatment baseline specimen is provided so that appropriate comparisons can be made between the pre- and post-transplant specimens. Since there is substantial variability between individuals in TREC copies, the best comparison is made to the patient's own baseline specimen rather than the reference range (which provides a guideline for TREC copies for age-matched healthy controls).

A consultative report will be generated for each patient.

### Cautions

While indicative of thymic function and T-cell recovery, T-cell receptor excision circle (TREC) results cannot be taken as a direct measure of thymic output because TRECs are diluted by peripheral T-cell division and intracellular degradation. In addition, the longevity of naive T cells in the periphery precludes TREC from being regarded as recent thymic emigrants. The assay provides a quantitative measure of TREC, ie, TREC copies per million CD3 T cells; however, this number should be regarded as a relative, rather than absolute, number because of the caveats explained above.

The TREC assay should not be ordered on adults over age 60 due to physiological decline in thymic function in the sixth and seventh decades of life.

Assay results are dependent on the patient's T-cell counts and in patients with profound lymphopenia it may be impossible to perform the assay if there are insufficient numbers of cells.
Temperature and time are critical to the performance of the assay. Temperatures that exceed or drop below 20 to 25

degrees C can dramatically affect the assay. High temperatures can cause substantial hemolysis that will interfere with the methodology used to perform the assay. Transportation delays may result in significant TREC degradation.

Consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets. See Clinical Information.

### Clinical Reference

1. Douek DC, McFarland RD, Keiser PH, et al: Changes in thymic function with age and during the treatment of HIV infection. Nature. 1998;396:690-694
2. Hazenberg MD, Verschuren MC, Hamann D, et al: T cell receptor excision circles as markers for recent thymic emigrants: basic aspects, technical approach, and guidelines for interpretation. J Mol Med. 2001;79:631-640
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7. Auletta JJ, Lazarus HM: Immune restoration following hematopoietic stem cell transplantation: an evolving target. Bone Marrow Transplant. 2005;35:835-857
8. Borghans JA, Bredius RG, Hazenberg MD, et al: Early determinants of long-term T cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency. Blood. 2006;108:763-769
9. Douek DC, Vescio RA, Betts MR, et al: Assessment of thymic output in adults after hematopoietic stem cell transplantation and prediction of T cell reconstitution. Lancet. 2000;355:1875-1881
10. Jamieson BD, Douek DC, Killian S, et al: Generation of functional thymocytes in the human adult. Immunity. 1999;10:569-575
11. Carmichael KF, Abayomi A: Analysis of diurnal variation of lymphocyte subsets in healthy subjects and its implication in HIV monitoring and treatment. 15th Intl Conference on AIDS, Bangkok, Thailand, 2004, Abstract B11052
12. Dimitrov S, Benedict C, Heutling D, et al: Cortisol and epinephrine control opposing circadian rhythms in T-cell subsets. Blood. 2009 May 21;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
15. Malone JL, Simms TE, Gray GC, et al: Sources of variability in repeated T-helper lymphocyte counts from HIV 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 assay involves pre-analytical preparation of a pure cell population followed by analytical evaluation of the DNA. A

modified peripheral blood mononuclear cells (PBMC) isolation is used to prepare a nearly pure population of CD3+ T cells (adults) or total lymphocytes (pediatrics) from whole blood. The resulting purity and cell counts are obtained from the TCD4 flow cytometric assay. The cells are then lysed with Proteinase K to a predetermined target concentration to release and expose the DNA for polymerase chain reaction (PCR). The genomic DNA and T-cell receptor excision circles (TREC) in the cell lysates are quantified in the real-time PCR assay, in triplicate, by using a fluorescent probe specific for the T-cell receptor delta-deletion TREC signal joint and a distinct fluorescent probe for the reference gene, albumin. There is one copy of TREC per CD3+ T cell, while there are 2 copies of albumin in every cell. A standard curve is used to determine the absolute quantity of TREC and albumin from the fluorescence intensities measured. The albumin counts are used to determine the cell counts in each reaction and to normalize the number of TREC copies to a standard reporting unit of copies per million CD3+ T cells. The pediatric TREC counts, though measured from total lymphocytes, can be adjusted to the same reporting units using the %CD3 purity from the flow cytometric assay.(Douek DC, Vescio RA, Betts MR, et al: Assessment of thymic output in adults after hematopoietic stem cell transplantation and prediction of T cell reconstitution. Lancet. 2000;355:1875-1881; Douek DC, Hill B: Personal Communication; 2005; Gaballa A, Clave E, Uhlin M, Toubert A, Arruda LCM: Evaluating thymic function after human hematopoietic stem cell transplantation in the personalized medicine era. Front Immunol. 2020 Jul 31;11:1341. doi: 10.3389/fimmu.2020.01341)

### PDF Report
No

### Day(s) Performed
Varies

### Report Available
6 to 8 days

### Specimen Retention Time
Extracted DNA: 2 months

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

## 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 and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
81479-Unlisted molecular pathology procedure

# Test Definition: TRECS

T-Cell Receptor Excision Circles Analysis, Blood

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| TRECS | TREC Analysis, B | In Process |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 615825 | TREC Copies | 62320-7 |
| 615822 | CD3 T Cells | 8122-4 |
| 615823 | CD4 T Cells | 24467-3 |
| 615824 | CD8 T Cells | 14135-8 |
| 616642 | Previous Run Date | 93126-1 |
| 616646 | Previous run TREC Copies | 93126-1 |
| 616643 | Previous run CD3 T Cells | 93126-1 |
| 616644 | Previous run CD4 T Cells | 93126-1 |
| 616645 | Previous run CD8 T Cells | 93126-1 |
| 615826 | Interpretation | 69047-9 |
| 615827 | Additional Information | 48767-8 |
| 615828 | Method | 85069-3 |
| 615829 | Disclaimer | 62364-5 |
| 615830 | Released By | 18771-6 |