

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 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 longitudinal assessment of thymic function and naive T-cell production. For comprehensive thymic function analysis on pediatric patients or posthematopoietic cell transplantation, order this test and CD4RT / CD4 T-Cell Recent Thymic Emigrants \(RTE\).](#)

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.

[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 draw specimen through a butterfly needle.
2. Send specimen in original tube. Do not aliquot.

Forms

[TREC Assay Patient Information](#) (T589) in Special Instructions

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 and Interpretive

Clinical Information

T cell reconstitution is a critical feature of the recovery 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 though 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 function can be determined by T-cell receptor excision circle (TREC) analysis. TRECs are extrachromosomal DNA byproducts of T-cell receptor (TCR) rearrangement, which are nonreplicative. TRECs are expressed only in T cells of thymic origin and each cell is thought to contain a single copy of TREC. Hence, TREC analysis provides a very specific assessment of T-cell recovery (eg, after hematopoietic cell transplantation) or numerical T-cell competence. There are several TRECs 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 PCR.

Clinical use of TRECs 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 TRECs in Hematopoietic Cell Transplantation (HCT) and Primary Immunodeficiencies (PID):

Following HCT, there is a period of prolonged immunodeficiency 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.(3-5) It has been shown that numerical T cell recovery is usually achieved by day 100 posttransplant, though there is an inversion of the CD4:CD8 ratio that can persist for up to a year.(4) 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-HCT.(6)

Recently, it has been shown in patients who received HCT for severe combined immunodeficiency (SCID) that T cell recovery early after stem cell transplant is crucial to long-term T cell reconstitution.(7) 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 TRECs early after HCT were most predictive for long-term reconstitution. This data suggests that frequent monitoring of T-cell immunity and TREC numbers after HCT can help identify patients who will fail to reconstitute properly, which would allow additional therapies to be instituted in a timely manner.(7) It would be reasonable to extrapolate such a conclusion to other diseases that are also treated by HCT.

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.(1,8,9) Thus, after HCT 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 HCT in adults.(8) In fact, this data shows that there is both a marked increase in the TREC numbers and a significant negative correlation of TREC copies with age posttransplant.

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 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 (NK) cell counts, on the other hand, are constant throughout the day.(10) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(11-13) 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,(14) and during summer compared to winter.(15) These data, therefore, indicate that timing and 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 (TRECs) generally show an inverse correlation with age, though there can be substantial variations in TREC copies relative to T-cell count within a given age group.

Following hematopoietic cell transplantation (HCT), 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 posttransplant treatment, this assay recommends that a pretransplant (prior to myeloablative or nonmyeloablative conditioning) or a pretreatment baseline specimen be provided so that appropriate comparisons can be made between the pre- and posttransplant treatment 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 TREC 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.

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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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
3. Parkman R, Weinberg K: Immunological reconstitution following hematopoietic stem cell transplantation. In *Hematopoietic Cell Transplantation*. Second edition. Edited by ED Thomas, KG Blume, SJ Forman. Blackwell

Scientific, Oxford, UK, 1999, pp 704-711

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7. 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
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9. Jamieson BD, Douek DC, Killian S, et al: Generation of functional thymocytes in the human adult. *Immunity* 1999;10:569-575
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11. 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
12. Dimitrov S, Lange T, Nohroudi K, Born J: Number and function of circulating antigen presenting cells regulated by sleep. *Sleep* 2007;30:401-411
13. 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
14. 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
15. Paglieroni TG, Holland PV: Circannual variation in lymphocyte subsets, revisited. *Transfusion* 1994;34:512-516

Performance

Method Description

This assay involves both preanalytical preparation of a pure cell population followed by analytical evaluation of the DNA. A modified peripheral blood mononuclear cells (PBMCs) 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 PCR. The genomic DNA and TREC in the cell lysates are quantified in the real-time PCR assay, in triplicate, by using a fluorescent probe specific for the TCR delta-deletion T-cell receptor excision circle (TREC) signal joint and a distinct fluorescent probe for the housekeeping gene, albumin. There is 1 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)

PDF Report

No

Day(s) and Time(s) Test Performed

Varies

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

Analytic Time

6 days

Maximum Laboratory Time

8 days

Specimen Retention Time

Extracted DNA: 2 months

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 and its performance characteristics 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

81479-Unlisted molecular pathology procedure

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
TREC	TREC, B	In Process

Result ID	Test Result Name	Result LOINC Value
28306	CD3 T Cells	8122-4
28307	CD4 T Cells	24467-3



Result ID	Test Result Name	Result LOINC Value
28308	CD8 T Cells	14135-8
87959	TREC Copies	62320-7
30292	Interpretation	69048-7
35200	Reviewed by	18771-6