

Overview

Useful For

Assessment of T-cell receptor diversity in various clinical contexts including primary immunodeficiencies, monitoring immune reconstitution posthematopoietic cell transplantation, and temporal assessment of repertoire changes in autoimmune diseases and viral infections

Testing Algorithm

Additional tests that could be ordered include:

- TREC / T-Cell Receptor Excision Circles (TREC) Analysis, Blood
- CD4RT / CD4 T-Cell Recent Thymic Emigrants (RTE)
- TCP / T-Cell Subsets, Naive, Memory, and Activated

Special Instructions

- [TCR V beta Spectratyping Assay Patient Information](#)

Method Name

Molecular TCR Vb-CDR3 Fragment Length Analysis

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Advisory Information

Mayo Laboratory Director/Consultant approval is required prior to ordering this test in patients >40 years of age.

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, it is recommended to perform specimen collection at the same time of day, if possible.

[Supplies: Ambient Shipping Box-Critical Specimens Only](#) (T668)

Specimen Type: Blood

Container/Tube: Lavender top (EDTA)

Specimen Volume:

Adults: 10 mL

Pediatrics:

-Preferred volume for >1 year: 3 mL

-Preferred volume for < or =1 year: 1 mL

Collection Instructions: Send specimen in original tube.

Forms

[TCR V beta Spectratyping Assay Patient Information](#) (T719) in Special Instructions

Specimen Minimum Volume

Adults: 5 mL

Pediatrics: 1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Other	Clotted

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

The rearrangement of the T-cell receptor (TCR) through somatic recombination of V (variable), D (diversity), J (joining), and C (constant) regions is a defining event in the development and maturation of a T cell. TCR gene rearrangement takes place in the thymus. During the process of rearrangement, DNA byproducts are generated called T-cell receptor excision circles (TREC) and these are used as markers of T cells that have recently emigrated from the thymus (TREC / T-Cell Receptor Excision Circles [TREC] Analysis, Blood). T cells, as part of the adaptive immune system, recognize foreign antigens when they are displayed on the surface of the body's own cells. T cells recognize these foreign antigens as peptides presented in the context of major histocompatibility complex (MHC) molecules through their T-cell receptors. Each TCR exists as 2 different polypeptide chains (heterodimers) called the TCR alpha chain and TCR beta chain, and these are linked by disulfide bonds. The majority of T cells (approximately 90%) in the body express TCRs with alpha and beta chains. A minority of T cells express other T-cell receptors

made of different polypeptide chains, gamma and delta. Each T cell has approximately 30,000 identical antigen receptors on its cell surface. A TCR has only 1 antigen-binding site, in contrast to the B-cell receptor, which has 2, and TCRs are never secreted and always remain on the cell surface. The alpha and beta chains are encoded by different gene loci (alpha and beta TCR gene locus). The beta chain locus rearranges before the alpha chain and a functional beta chain has to be produced in order for the T cell to form a pre-T-cell receptor. The expression of the rearranged beta chain with an alpha chain precursor suppresses additional gene rearrangement at the TCR beta locus. The TCR alpha chain locus rearrangement can proceed even with production of a functional alpha chain until there is positive selection of the particular T cell. However, it is important to note that each T cell has a single functional specificity for its TCR.

A key concept in understanding the immune response is that there is enormous diversity in the immune system to enable protection against a huge array of pathogens. Since the germline genome is limited in size, diversity is achieved not only by the process of V(D)J recombination but also by junctional (junctions between V-D and D-J segments) deletion of nucleotides and addition of pseudo-random, nontemplated nucleotides. In particular, the CDR3 (complementarity determining region 3), which is the most critical determinant of antigenic specificity in T cells (and also B cells) is short (between 66-90 nucleotides, approximately 20-30 amino acids) and amenable to assessment of length by fragment length analysis, which provides a size resolution of up to 1 base pair between different CDR3 regions. It is thought that the CDR3-TCR beta chain repertoire in healthy adults contains somewhere between 3 and 4 million unique sequences.(1) Other reports suggest that the unique TCR repertoire after thymic selection is between 10 to 100 million in humans.(2) There is, however, a bias in TCR selection with overrepresentation of certain TCRs that are widely used in individuals who share the same major histocompatibility (MHC) types and these are called "public TCRs." Public TCRs generally have fewer random nucleotide additions in their sequence. The TCR V beta repertoire varies significantly between individuals and populations because of 7 frequently occurring inactivating polymorphisms in functional gene segments and a large insertion/deletion-related polymorphism encompassing 2 V beta gene segments. With this latter situation, the TCR Vb 6-2/6-3 and TCR Vb 4-3 genes are frequently deleted from all ethnic groups.(3) It has been reported that the total number of functional TCR V beta gene segments expressed by an individual varies from 42 to 47.(4)

Deep sequencing technologies are evolving to analyze this large diversity in the adaptive immune receptors,(5,6) however; deep sequencing of the T-cell and B-cell receptor genes is not yet widely available and is expensive. Flow cytometry-based analysis to assess TCR V beta diversity is available; however, the antibodies are limited and therefore the assay is not capable of assessing the entire TCR V beta repertoire. On the other hand, TCR beta chain repertoire analysis by fragment length analysis (spectratyping) using fluorescent primers to measure CDR3 length variability, while unable to provide the extreme high resolution of deep sequencing, can provide a global "snapshot" of T-cell receptor repertoire diversity, which is useful for most clinical applications where this level of assessment is required.(7-14) It is important to note that this method uses PCR to amplify the rearranged variable regions to provide adequate template for sequencing (fragment length analysis), and this can introduce bias due to the more efficient amplification of certain templates compared to others. However, despite this limitation, since this assay is not quantitative, it is still able to provide an assessment of diversity by measuring the CDR3 length in various TCR V beta genes, which are organized into 24 families.

Reference Values

Reference values will be provided in the patient report.

Interpretation

An interpretive report will be provided with adult and pediatric reference values for the relative contribution of each family to the total repertoire (% diversity ratio). The interpretation will be based on visual analysis of the spectratype (polyclonal, oligoclonal, or monoclonal) for each family as well as assessment of the number of peaks (numerical value not reported), and diversity ratio (DR) (reported value). Information on the distribution of peaks, eg, Gaussian vs non-Gaussian, will also be included in the report, where appropriate. Internal analytical and quality controls will be assessed to determine the suitability of reporting a patient result. Correlation with the clinical context will be made when possible, based on clinical history provided in the patient information sheet (which should be provided with the

patient sample).

Cautions

This is essentially a qualitative/semiquantitative assay, with the diversity ratio (calculated as described in method description), and visual analysis of the spectratype. This assay does not quantify in any way the amount of transcript for each T-cell receptor. This assay is **not** a deep sequencing assay and, therefore, does not provide the granularity of information offered by deep sequencing.

This assay is intended to generate a spectratype (immunoscope) of the T-cell receptor V beta repertoire and to draw inferences on repertoire diversity based on the number and distribution of peaks across the 24 TCR V beta families.

If the CD3+ T-cell count is less than 70 cells/mL, at least 2 to 3 mL blood may be needed to obtain enough cells to perform the test. For this reason, if only 1 mL blood is provided, this test should not be ordered in patients with completely absent or less than 70 T cells/mL blood due to underlying disease or treatment, as there may be inadequate sample to amplify TCR V beta families in contexts of such profound T-cell lymphopenia.

This test should ideally not be ordered in patients over the age of 40 years without prior discussion with laboratory directors on clinical utility and interpretation in specific clinical contexts.

Clinical Reference

1. Robins HS, Campregher PV, Srivastava SK, et al: Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. *Blood* 2009;114:4099-4107
2. Arstila TP, Casrouge A, Baron V, et al: A direct estimate of the human alpha-beta T cell receptor diversity. *Science* 1999;958-961
3. Brennan RM, Petersen J, Neller MA: The impact of a large and frequent deletion in the human TCR beta locus on antiviral immunity. *J Immunol* 2012;188:2742-2748
4. Mackelprang R, Carlson CS, Subrahmanyam L, et al: Sequence variation in the human T-cell receptor loci. *Immunol Rev* 2002;190:26-39
5. Warren EH, Matsen IV FA, Chou J: High-throughput sequencing of B- and T-lymphocyte antigen receptors in hematology. *Blood* 2013;122:19-22
6. Robins H: Immunosequencing: applications of immune repertoire deep sequencing. *Curr Opin Immunol* 2013;25:646-652
7. Gorski J, Yassai M, Zhu X, et al: Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 spectratyping. *J Immunol* 1994;152:5109-5119
8. Memon SA, Sportes C, Flomerfelt FA, et al: Quantitative analysis of T cell receptor diversity in clinical samples of human peripheral blood. *J Immunol Methods* 2012;375:84-92
9. van Heijst JMJ, Ceberio I, Lipuma LB, et al: Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. *Nature Medicine* 2013;19:372-378
10. Wada T, Schurman SH, Garabedian EK, et al: Analysis of T-cell repertoire diversity in Wiskott-Aldrich syndrome. *Blood* 2005;106:3895-3897
11. Pirovano S, Mazzolari E, Pasic S, et al: Impaired thymic output and restricted T-cell repertoire in two infants with immunodeficiency and early-onset generalized dermatitis. *Immunol Letters* 2003;86:93-97

12. Villa A, Notarangelo LD, Roifman CM: Omenn syndrome: inflammation in leaky severe combined immunodeficiency. *J Allergy Clin Immunol* 2008;122:1082-1086

13. Sullivan KE: The clinical, immunological and molecular spectrum of chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. *Curr Opin Allergy Clin Immunol* 2004;4:505-512

14. Markert ML, Devlin BH, McCarthy EA: Thymus transplantation. *Clin Immunol* 2010;135:236-246

Performance

Method Description

CD3+ T cells are enriched and purified from EDTA whole blood. RNA is obtained from the T cells and converted into cDNA to maximize sample stability. The cDNA is amplified using the polymerase chain reaction (PCR), during which the different CDR3 fragment lengths of the TCR Vb families are fluorescently labeled. The pool of varying CDR3 fragment lengths are separated by size on a capillary electrophoresis genetic analyzer. As the fluorescent label of each CDR3 fragment passes through the laser, the size and fluorescent intensity is recorded. The resulting image is a cluster of fluorescent peaks with single-base-pair separation and different fluorescent intensities, approximately corresponding to the number of fragments of that size represented in the patient's original RNA. The peak patterns are reviewed for organization (number of peaks), relative intensity across peaks, and size distribution. The number of individual peaks is compared to a reference range established from over 140 healthy donors equally represented by both genders and across the pediatric and adult age spectrum. The reporting units are normalized among the patient population by using a diversity ratio for each TCR V beta family. The diversity ratio for each Vb family is determined by the number of peaks in that specific family relative to all peaks within the patient's sample expressed as a percentage. The analytical process in the laboratory utilizes a variety of controls to assess the performance of the assay and reliability of the result provided. The fragment length analysis is performed by the Gene Marker software and the spectratype is assembled for interpretation. An interpretive report will be provided for each patient sample and includes information on the diversity ratio for each family. The spectratype will be made available on request of the physician. (Unpublished Mayo method)

PDF Report

Supplemental

Day(s) and Time(s) Test Performed

Varies

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

Analytic Time

10 days

Maximum Laboratory Time

14 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

81340-TRG (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s); using amplification methodology (eg, polymerase chain reaction)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
TCRVB	TCR Spectratyping, B	In Process

Result ID	Test Result Name	Result LOINC Value
36473	Family 2 Diversity Ratio	82501-8
36474	Family 3-1 Diversity Ratio	82500-0
36475	Family 4 Diversity Ratio	82499-5
36476	Family 5 Diversity Ratio	82498-7
36477	Family 6 Diversity Ratio	82497-9
36478	Family 7 Diversity Ratio	82496-1
36479	Family 9 Diversity Ratio	82495-3
36480	Family 10 Diversity Ratio	82494-6
36481	Family 11 Diversity Ratio	82493-8
36482	Family 12 Diversity Ratio	82492-0
36483	Family 13 Diversity Ratio	82491-2
36484	Family 14 Diversity Ratio	82490-4
36485	Family 15 Diversity Ratio	82489-6
36486	Family 16 Diversity Ratio	82488-8
36487	Family 18 Diversity Ratio	82487-0
36488	Family 19 Diversity Ratio	82486-2
36489	Family 20-1 Diversity Ratio	82485-4
36490	Family 24-1 Diversity Ratio	82484-7
36491	Family 25 Diversity Ratio	82483-9
36492	Family 27 Diversity Ratio	82482-1
36493	Family 28 Diversity Ratio	82481-3
36494	Family 29 Diversity Ratio	82480-5

Result ID	Test Result Name	Result LOINC Value
36518	Family 30 Diversity Ratio	82479-7
36495	Interpretation	69047-9
36496	Reviewed by	18771-6