Test Definition: TCRB

T-Cell Receptor V-Beta Repertoire Analysis, Spectratyping, Blood

Overview

Useful For

Assessment of T-cell receptor diversity in various clinical contexts including inborn errors of immunity (formerly primary immunodeficiencies), monitoring immune reconstitution post-hematopoietic stem cell transplantation, and temporal assessment of repertoire changes in autoimmune diseases and viral infections

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

Ordering Guidance

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

Additional Testing Requirements

Additional tests that could be ordered in conjunction with this test include:
-TRECS / T-Cell Receptor Excision Circles (TREC) Analysis, Blood
-CD4RT / CD4 T-Cell Recent Thymic Emigrants, Blood
-TCP / T-Cell Subsets, Naive, Memory, and Activated, 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 and/or on observed holidays may be canceled.

Necessary Information

Ordering physician's name and phone number are required.

TCR V beta Spectratyping Assay Patient Information (T719) **is required**. Testing will proceed without the form; however, results will be held under 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)
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 whole blood specimen in original tube. **Do not aliquot.**

Forms

TCR V beta Spectratyping Assay Patient Information (T719) **is required**.

Specimen Minimum Volume

Adults: 5 mL
Pediatrics: 1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Clotted	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

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 (TRECS / T-Cell Receptor Excision Circles 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 TCRs. 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 one antigen-binding site, in contrast to the B-cell receptor, which has two. 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, non-templated nucleotides. In particular, the CDR3 (complementarity determining region 3), which is the most critical determinant of antigenic specificity in T cells (and 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 one 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 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 alterations (ie, polymorphisms) in functional gene segments and a large deletion/insertion-related alteration 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 cannot assess 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 TCR 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 polymerase chain reaction 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. 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

References 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.

The assay is not validated for determining clonality in the context of hematologic malignancies.

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/mcL, 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/mcL 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 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, Subrahmanyan 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
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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 complementary DNA (cDNA) to maximize sample stability. The cDNA is amplified using the polymerase chain reaction (PCR), during which the different CDR3 fragment lengths of the T-cell receptor (TCR) V beta families are fluorescently labeled. The pool of varying CDR3 fragment lengths is 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 sexes 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 V beta 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) Performed
Varies

Report Available
20 to 24 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
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
TCRB	TCRVB Spectratyping, B	21751-3

Result ID	Test Result Name	Result LOINC® Value
616418	CD3 T Cells	8122-4
616419	CD4 T Cells	24467-3
616420	CD8 T Cells	14135-8
615831	Family 2 Diversity Ratio	82501-8
615832	Family 3-1 Diversity Ratio	82500-0
615833	Family 4 Diversity Ratio	82499-5
615834	Family 5 Diversity Ratio	82498-7
615835	Family 6 Diversity Ratio	82497-9
615836	Family 7 Diversity Ratio	82496-1
615837	Family 9 Diversity Ratio	82495-3

615838	Family 10 Diversity Ratio	82494-6
615839	Family 11 Diversity Ratio	82493-8
615840	Family 12 Diversity Ratio	82492-0
615841	Family 13 Diversity Ratio	82491-2
615842	Family 14 Diversity Ratio	82490-4
615843	Family 15 Diversity Ratio	82489-6
615844	Family 16 Diversity Ratio	82488-8
615845	Family 18 Diversity Ratio	82487-0
615846	Family 19 Diversity Ratio	82486-2
615847	Family 20-1 Diversity Ratio	82485-4
615848	Family 24-1 Diversity Ratio	82484-7
615849	Family 25 Diversity Ratio	82483-9
615850	Family 27 Diversity Ratio	82482-1
615851	Family 28 Diversity Ratio	82481-3
615852	Family 29 Diversity Ratio	82480-5
615853	Family 30 Diversity Ratio	82479-7
615854	Interpretation	69047-9
615855	Additional Information	48767-8
615856	Method	85069-3
615857	Disclaimer	62364-5
615858	Released By	18771-6