

**Performing Site:**

Mayo Clinic Laboratories - Rochester Main Campus
200 First Street SW, Rochester MN 55905

Phone: 800-533-1710

Lab Director: William G. Morice, II, M.D., Ph.D.

CLIA Certificate: 24D0404292

TESTINGRNA, ATLAS N**MEDICAL RECORD # (PATIENT ID) SA00150980**

DOB 03/03/2021
SEX Male
CLIENT MRN SA00150980
REQUESTED BY CLIENT CLIENT

CLIENT ID/WARD 7028846
CLIENT/NAME WARD MCL RochesterCampus
CITY, ST, ZIP Rochester
MN 55901

ORDER # L604000120
CLIENT ORDER # SA00150980
DATE COLLECTED 3/4/2022 8:00 AM
DATE RECEIVED 3/4/2022 8:38 AM
DATE REPORTED 3/7/2022 5:28 PM

TCRVB Spectratyping, B

Result Name	Result	Flag	Units	Reference Values
CD3 T Cells	2807	Normal	cells/mcL	1484-5327
CD4 T Cells	1901	Normal	cells/mcL	733-3181
CD8 T Cells	800	Normal	cells/mcL	370-2555
Family 2 Diversity Ratio	3	Normal	%	>=3
Family 3-1 Diversity Ratio	3	Normal	%	>=0
Family 4 Diversity Ratio	4	Normal	%	>=3
Family 5 Diversity Ratio	4	Normal	%	>=2
Family 6 Diversity Ratio	7	Normal	%	>=4
Family 7 Diversity Ratio	14	Normal	%	>=11
Family 9 Diversity Ratio	3	Normal	%	>=3
Family 10 Diversity Ratio	7	Normal	%	>=4
Family 11 Diversity Ratio	4	Normal	%	>=2
Family 12 Diversity Ratio	3	Normal	%	>=3
Family 13 Diversity Ratio	4	Normal	%	>=2
Family 14 Diversity Ratio	3	Normal	%	>=3
Family 15 Diversity Ratio	3	Normal	%	>=2
Family 16 Diversity Ratio	2	Normal	%	>=0
Family 18 Diversity Ratio	3	Normal	%	>=3
Family 19 Diversity Ratio	4	Normal	%	>=3
Family 20-1 Diversity Ratio	4	Normal	%	>=3
Family 24-1 Diversity Ratio	4	Normal	%	>=3
Family 25 Diversity Ratio	5	Normal	%	>=2
Family 27 Diversity Ratio	4	Normal	%	>=0
Family 28 Diversity Ratio	4	Normal	%	>=3

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Result Name	Result	Flag	Units	Reference Values
Family 29 Diversity Ratio	4	Normal	%	>=2
Family 30 Diversity Ratio	4	Normal	%	>=3

Interpretation

Interpretation: This result shows an essentially normal TCR Vb repertoire. Evaluation of 23 families with 28 probes shows 26 with a polyclonal Gaussian distribution, 1 with a polyclonal non-Gaussian distribution, and 1 with an oligoclonal distribution; oligoclonality is observed frequently in family 16 in this assay. The diversity ratio (DR) for all families is within normal limits. The diversity ratio (DR) has to be interpreted in the context of the visual analysis of the spectratype since it cannot differentiate between polyclonal Gaussian versus non-Gaussian distribution. T cell enumeration shows normal CD3+ total T cell, CD4+ T cell and CD8+ T cell counts.

Clinical Context: Recurrent hospitalisations with upper respiratory infection causing respiratory distress, enteroviral meningitis, poor growth, genetic testing pending.

Sample Information: The sample provided is a baseline analysis of the TCR Vb repertoire.

Method:

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)

Disclaimer:

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

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specific clinical contexts.

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.

Released By:

Amir A. Sadighi Akha, M.D., D. Phil., D(ABMLI)

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TCRVβ Spectratyping Patient Report

TEST NGRNV

Patient Name ATLAS

Patient SOF I Lab Order # 22-8 443

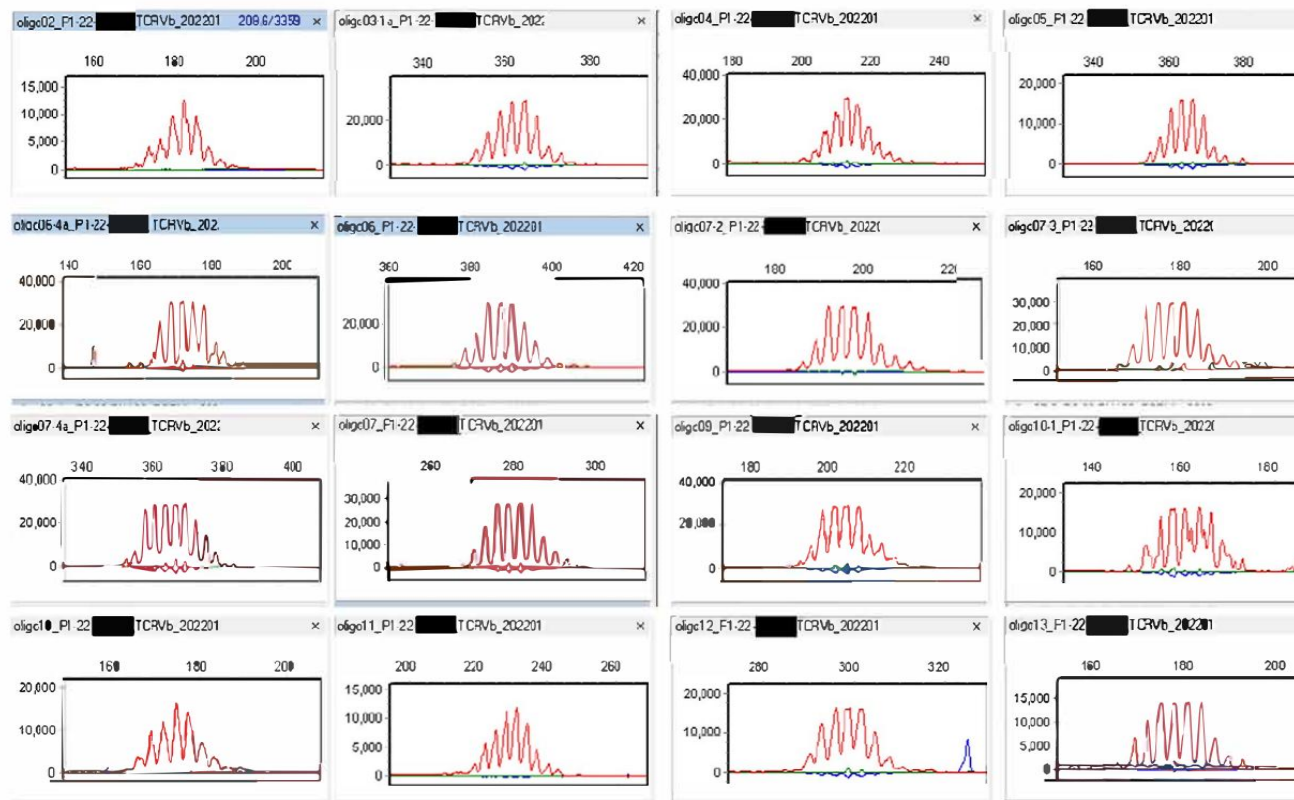
Patient Order # L601000195

Patient DOB 1/15/2021

Patient Gender F

Specimen Date 1/24/2022

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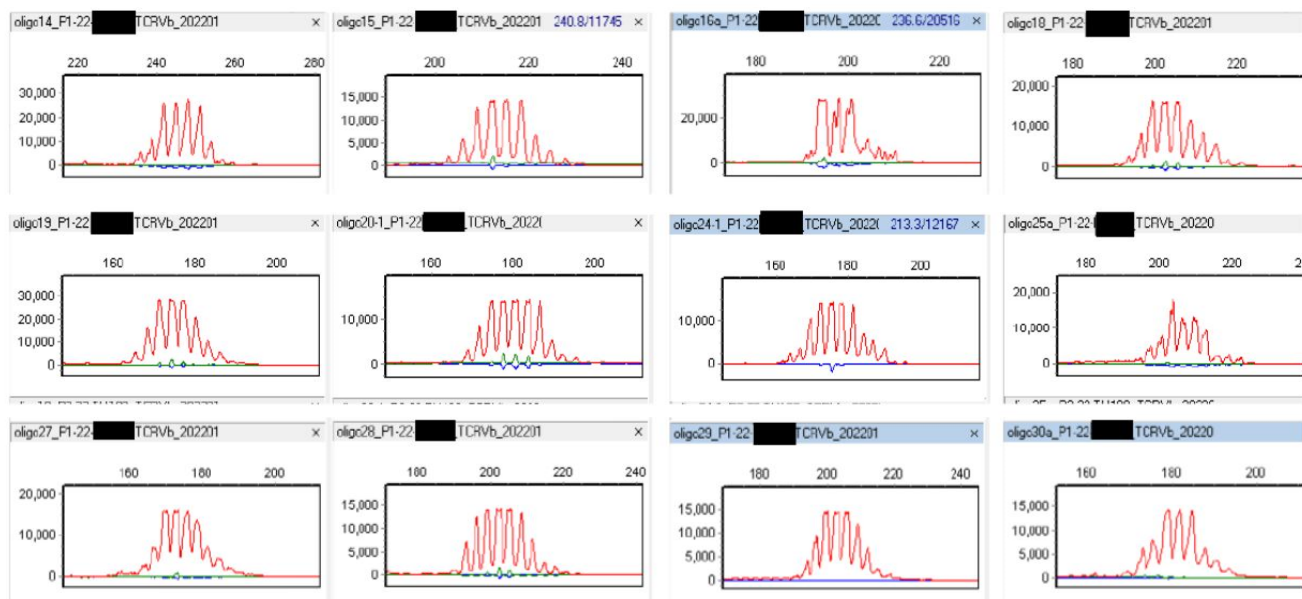
Please note: For clarity, the designation "oligo" is used on spectratype data files, to distinguish among sub-families. All "oligos" of the same family (example: 7-2, 7-3, 7-4 and 7) are combined to generate the reported diversity ratio for each family.

TCRVβ Spectratyping Patient Report

TEST NGRNV,
Patient Name ATLAS
Patient SOFTLab Order # 22-7930
Patient Order # L601000195

Patient DOB 1/15/2021
Patient Gender F
Specimen Date 1/24/2022

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All "oligos" of the same family (example: 7-2, 7-3, 7-4 and 7) are combined to generate the reported diversity ratio for each family.