PrismRA® Technical Specifications

Scipher Medicine Corporation
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Intended Use

The Scipher Medicine PrismRA®, a molecular signature response classifier (MSRC) blood-based test, is used to determine a patient's likelihood of inadequate response to tumor necrosis factor-α inhibitor (TNFi) therapy. PrismRA is intended for use by advanced healthcare providers treating patients diagnosed with rheumatoid arthritis (RA) who are 18 years or older, have a history of failure, contraindication or intolerance to at least one csDMARD and are in moderate or high disease activity that are either:

1) naïve to a biologic or targeted synthetic disease modifying antirheumatic drug (b/tsDMARD); OR

2) currently on a TNFi; and are considering adjusting the dose, starting or switching to a different b/tsDMARD.

Summary and Explanation: PrismRA evaluates RNA expression data from whole blood samples, demographic variables, clinical and laboratory metrics to detect a molecular signal of inadequate response (defined as achieving ACR50 at 6 months) to all TNFi therapies for patients with RA.

PrismRA evaluates 19 gene expression features, sex, body mass index (BMI), patient global assessment (PtGA), and anti-CCP.

The PrismRA result is an integer score that represents the likelihood of inadequate response to TNFi therapies in that patient. The 23 biomarkers are integrated into a single predictive model that generates a score on a scale of 1 to 25 that represents the likelihood of inadequate response to TNFi therapies.

Test Methodology: PrismRA is performed as a laboratory-developed test using RNA extracted from PAXgene® whole blood sample, and anti-CCP analyses from a serum separation tube on a Cobas® 6000 (Roche Diagnostics). PAXgene specimens are processed using the MagMax™ for Stabilized Blood PAXgene Tubes RNA Isolation Kit. The extracted RNA samples are quantified with M200 absorbance. RNA integrity is determined per Agilent TapeStation or Bioanalyzer operating procedures. RNA samples (100-1000ng) are processed using the KAPA RNA HyperPrep™ Kit with RiboErase (HMR) Globin (KAPA/Roche). After library amplification, samples are quantified using Agilent D1000 reagents per the Agilent TapeStation or Bioanalyzer operating procedures. Samples are sequenced on a NovaSeq™ 6000 (Illumina).

Libraries are sequenced to high uniform depth (targeting > 7 million protein coding reads). Sequence data are then processed using a customized analysis pipeline designed to determine gene expression across the genome.

Interpretive Criteria

The lower the PrismRA Score, the less likely the patient will have an inadequate response to TNFi therapy. However, a low PrismRA score does not ensure a positive response to TNFi therapies.

PrismRA Scores <10.6 = no molecular signature: A patient with a PrismRA Score <10.6 does not have a detectable molecular signal of inadequate response to TNFi therapies. In a PrismRA validation study, the observed response rate to TNFi therapies for such individuals was greater than that of the unstratified patient population.[1]

PrismRA Scores ≥10.6 = a high likelihood of inadequate response to TNFi therapies: A patient with a PrismRA Score ≥10.6 has a high signal of inadequate response to TNFi therapies. This corresponds to an approximate 90% chance of inadequately responding to TNFi therapies. Thus, the patient has approximately 10% chance of responding to TNFi therapies.

PrismRA Scores ≥18.5 = a very high likelihood of inadequate response to TNFi therapies: A patient with a PrismRA Score ≥18.5 has a very high signal of inadequate response to TNFi therapies. This corresponds to an approximate 95% chance of inadequately responding to TNFi therapies. Thus, this patient has an approximate 5% chance of responding to TNFi therapies.

Limitations

Contraindications: The PrismRA test is a blood draw, thus contraindications are those consistent with routine phlebotomy. The major contraindications for phlebotomy are skin conditions like cellulitis or abscesses that would cause direct seeding of infectious agents (like bacteria) into the blood. Other complications that should be considered are the presence of vascular access devices in hospitalized patients, vascular grafts, bleeding under the skin, and if the patient has palpable venous fibrosis.

Warnings and Precautions

- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- PrismRA is not intended for use in patients younger than 18 years of age.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences in accordance with the standard-of-care.
- This test, and the interpretive content in the PrismRA report, were developed and the performance characteristics were determined by Scipher Medicine Corporation. The test and report have not been reviewed, cleared, or approved by the Food and Drug Administration (FDA). The PrismRA test (including the interpretive content in the PrismRA report) is a Laboratory Developed Test from a CLIA-certified (34D2180776) and CAP-accredited (8821838) laboratory.

Performance Characteristics

Clinical Validation: In a prospective, blinded validation cohort, the molecular signature response classifier identified likely inadequate responders with a positive predictive value (PPV) of 87.7% (95% CI: 78-94%), sensitivity of 60.2% (95% CI: 50-69%), and specificity of 77.3% (95% CI: 65-87%) among b/tsDMARD-naïve and TNFi-exposed patient samples.[2]

The performance characteristics of PrismRA among b/tsDMARD-naïve patient samples are a PPV of 86.2% (95% CI: 73-95%), sensitivity of 67.8% (95% CI: 54-79%), and specificity of 70.7% (95% CI: 54-84%). The performance characteristics of PrismRA among TNFi-exposed patient samples are a PPV of 92.0% (95% CI: 75-99%), sensitivity of 51.0% (95% CI: 36-66%), and specificity of 88.0% (95% CI: 69-97%).[2]

Precision and Reproducibility: The precision and reproducibility data demonstrated a high level of concordance among the PrismRA results for inter-assay reproducibility (100%) and intra- assay reproducibility (92.6%). The overall precision was 95.8%. The analytical sensitivity of the PrismRA test is 93.1%.[2]

Interference: Although rare, PrismRA test results and clinical interpretation may be impacted by other factors not addressed above.

Sequential Testing: Data does not support the sequential testing of PrismRA for a patient that has already been successfully tested.

Quality Control Measures

Minimum RNA sample quality control metrics:

- TapeStation RIN ≥ 4.1
- Concentration ≥ 10 ng/µL
- Library yield ≥ 10 nM

Minimum RNA sequencing run quality control metrics:

- Perfect index: >85%
- Bases over Q30: >75%
- Mean quality score: >30

Minimum RNA sequencing quality control metrics required prior to PrismRA bioinformatics pipeline assessment:

- FASTQ files are properly formatted with appropriate header lines, separator lines and a uniform base pair length and quality score length for every read.
- Uniform base pair length and quality score length for every read
- Median Phred score >25 and a lower quartile >10 for all bases
- Mean Phred score for every tile <(Mean-2) Phred score for that base across all tiles
- The most frequent observed mean Phred score >27
- Protein coding reads >7 million

Sample rejection criteria: Inappropriate sample types can cause cancelation of the test. Inappropriate sample types include: samples from patients not diagnosed with RA, samples collected in expired tubes, unlabeled or incorrectly labeled samples, samples for which insufficient clinical information has been provided, samples of insufficient RNA quality (RNA integrity number <4), samples of insufficient RNA quantity (<7 million protein coding reads). Insufficient quantity or quality may be due to inadequate PAXgene® tube inversions, damage occurring during shipping, an extended period of time between sample collection and receipt by the laboratory.

Sample Collection

Description of method: PrismRA is tested on blood samples; a PAXgene tube used for next-generation sequencing of RNA and a serum separation tube used to collect serum for anti-CCP testing. All sample collection tubes are provided in a barcoded PrismRA kit. The blood samples must be collected following the applicable instructions of the manufacturer. The PAXgene tubes must be the last tubes drawn. Samples must be shipped to the Scipher Medicine Laboratory no later than 24 hours after the blood draw. PrismRA was developed and its performance characteristics were determined by Scipher Medicine.

PrismRA sample collection test kit contents: The PrismRA test kit includes a sample shipping kit that is sent to ordering laboratories. The shipping kit contains the following components:

- One serum separation tube
- Two PAXgene Blood RNA tubes
- Absorbent sleeve
- 3oz. gel wrap
- Test requisition form
- 5 labels for blood tubes (3 for blood sample tubes, 2 extra if needed)
- 1 label for the test requisition form
- Insulated foil envelope
- Biohazard zip poly bag
- Clinical Pak with pre-affixed return shipping label

All other reagents, materials and equipment needed to perform the assay are used in the Scipher Medicine Laboratory.

Test Ordering: The PrismRA kit comes with a test requisition form, which must be fully completed and signed by the ordering physician or other authorized medical professional. In this form, information must be provided, including provider information, patient information, ICD-10 code(s), the test being requested, and billing information. A copy of the front and back of the policyholder's insurance card and a patient demographic face sheet must be attached as well. Furthermore, the phlebotomist must fill out a specific section of the requisition form. The completed form should be placed in the PrismRA collection kit to be returned to Scipher Medicine with the three blood tubes.

Sample Collection: Blood will be drawn through standard manufacturer's instructions, using PAXgene tubes for RNA analyses and serum separation tubes for anti-CCP analyses.

Sample collection instructions are as follows:

1. The tubes must first be labeled before any sample collection. They must be labeled with the patient's full name, date of birth, and the date of the blood draw on the three barcode stickers provided. Two PAXgene Blood RNA tubes and one serum separation tube must be labeled with these stickers.
2. The test requisition form must be completed, and all required fields must be filled out. The barcode label should be affixed to the form.
3. The blood is then drawn through the attachment of a 21g or 23g butterfly needle set. The serum separation tube must be drawn first, then the PAXgene tubes MUST be drawn last. If the vein fails and a redraw is needed, you must use another butterfly needle and draw a discard tube prior to drawing the PAXgene tube. If a new tube(s) is needed, the contents from an additional PrismRA kit must be used. All tubes must be ensured to be labeled with the appropriate tube labels from the original kit. The sample tubes should NOT be shaken. The tube should be completely filled.
4. The tubes should be gently inverted 8-10 times immediately upon blood draw. The blood must be completely mixed with the PAXgene tube stabilization reagent, so the final color of the sample is uniform. Sample tubes should NOT be shaken.
5. Place the PAXgene tubes upright in a test tube rack at ambient (18-25° C) for a minimum of 2 hours. Do NOT spin the PAXgene tubes. Place the SST tube upright in a test tube rack at ambient (18-25° C) for a minimum of 30 minutes. If possible after the waiting period. SPIN JUST THE SST TUBE at the speed of 1000 to 1300 RCF for 10 minutes in a swinging bucket centrifuge or 15 minutes in a fixed-angle centrifuge.
6. The tubes are packaged as follows:
    a. The three tubes are placed in an absorbent sleeve.
    b. The sleeve and 3 oz. gel wrap are placed in an insulated envelope.
    c. The envelope is placed in a biohazard zip poly bag.
    d. The bag is placed into the kit box.
    e. The completed test requisition form is added to the kit, and the box is closed.
    f. The kit box is placed in a pre-labeled FedEx Clinical Pak.
7. The FedEx Clinical Pak is shipped overnight to Scipher Medicine Laboratory. Samples must be shipped no longer than 24 hours after the blood draw, and samples must not be shipped on Saturdays.

Sample preparation: Total RNA is extracted from the blood sample and sequenced utilizing validated methods. Serum anti-CCP measurements are performed at Scipher Medicine Laboratory using validated methods. Final algorithmic analysis is performed at Scipher Medicine Laboratory.

Sample Stability: Blood will be drawn according to standard manufacturer's instructions, using PAXgene tubes for RNA analysis and serum separation tubes for anti-CCP analysis. PAXgene blood tubes contain a proprietary reagent for stabilization of intracellular RNA immediately upon collection. PAXgene blood RNA tubes can remain stabilized for up to 3 days at room temperature (18-25° C), up to 5 days at 2-8° C, and 11 years at -20° C or -70° C. Serum separation tubes contain a clot activator and a barrier polymer that has a density that causes it to move upward during centrifugation to the serum-clot interface. The serum separation tubes are stable up to 1 week when refrigerated. For prolonged storage, the separated serum should be kept frozen in a new tube at -20° C or lower.

Turnaround time: The turnaround time is approximately 5-7 business days from the blood draw receipt to the report being delivered to the ordering physician.

1. Cohen S, Wells AF, Curtis JR, et al. A Molecular Signature Response Classifier to Predict Inadequate Response to Tumor Necrosis Factor-α Inhibitors: The NETWORK-004 Prospective Observational Study. *Rheumatol Ther.* 2021 Sep;8(3):1159-1176. doi: 10.1007/s40744-021-00330-y. **2.** Jones A, Rapisardo S, Zhang L, et al. Analytical and clinical validation of an RNA sequencing-based assay for quantitative, accurate evaluation of a molecular signature response classifier in rheumatoid arthritis. *Expert Rev Mol Diagn.* 2021 Nov;21(11):1235-1243. doi: 10.1080/14737159.2021.2000394.