Rady Children's Institute Genomic Medicine | Clinical Genome Center

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CAP ID# 9487427

Whole Genome Sequencing Test Definition

This document outlines test specifications for Whole Genome Sequencing (WGS) at the Rady Children's Institute for Genomic Medicine Clinical Genome Center (RCIGM CGC). This test is a laboratory developed test (LDT) that adheres to the guidelines established by CLIA-'88. The WGS test must be ordered by a licensed physician.

Indications for Testing

Indication for performing the WGS test includes:

- Molecular diagnosis of suspected genetic disease
- Critically-ill infants in the intensive care unit with no unifying diagnosis
- Identification of rare genetic variants in patients who have failed to receive a diagnosis despite extensive work-up and testing

Test Type

Laboratory Developed Test (LDT)

Turn Around Time (Tat) Definitions

TAT is defined as the duration from when a Test is accepted to the time results are signed out and reported.

Operational definitions for WGS TAT for the FINAL report are as follows:

- Ultra-Rapid: 7-14 days (Preliminary positive reports within 3 calendar days)
- Rapid: 7-14 days (Preliminary positive reports within 5 calendar days)
- Standard: Less than 30 days

Background and Methodology

Sequence via next generation sequencing (NGS) technology is generated from genomic DNA. PCR-free library preparation is performed prior to whole genome sequencing (WGS). An average genomic coverage of at least 35x is obtained for each proband genome. Alignment and variant calling are performed using the Edico DRAGEN pipeline using the official reference build 37.1. Copy number variation (CNV) calling is performed using a combination of CNV callers. Interpretation of CNVs is focused on variants that overlap or have a boundary that lies within 1 kb of an exon in one of approximately 8000 genes known to have a gene-disease association. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, and larger deletions and duplications. Likely pathogenic and pathogenic reported variants may be confirmed using orthogonal technologies in instances where the variant does not meet internal RCIGM quality control thresholds.

A phenotype-driven analysis is performed. Therefore, only variants related to the indication for testing are reported. Incidentally ascertained medically-actionable findings are also reported unless the individual has opted out of receiving incidental results. Likely pathogenic and pathogenic variants that may explain the patient's phenotype will be reported. Selected variants of uncertain significance may be reported as well. Reported variants are curated and classified in accordance with the American College of Medical Genetics and Genomics Guidelines (Richards et al. 2015, PMID: 25741868; Kearney et al. 2011, PMID 21681106; South et al. 2013, PMID 24071793).

The sensitivity and specificity for SNVs (single nucleotide variants) and small insertions and deletions up to 50 base pairs is greater than 99%. The analytical validity of SNVs was assessed using reference samples provided through the Genome in a Bottle (Zook et al. 2019, PMID: 30936564).

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The sensitivity for larger deletions and duplications from WGS is estimated to be greater than 85%, although reliable reference data for these types of events are not well established. Deletions and duplications from 1 Kb to whole chromosomal abnormalities are detected with this test.

This test is validated for copy number analysis of exons 7 and 8 of the SMN1 and SMN2 genes. Over 95% of pathogenic variation for SMA involves biallelic loss of exon 7 of the SMN1 gene (Prior et al. 2010, PMID: 20057317).

Other variation within the SMN1 gene is not detected with this assay. Results will only be reported in the proband if 0 copies of SMN1 are detected. Parental carrier status will be reported for affected patients if samples are available. Results are orthogonally confirmed by Multiplex Ligation-dependent Probe Amplification (MLPA). Whole genome sequencing is unable to determine the phase of SMN1 variants in the absence of parental testing. Therefore, in the absence of phasing this assay does not exclude the possibility than an individual harbors two copies of SMN1 on the same allele and no copies on the other allele (silent carriers), two pathogenic sequence variants on the same SMN1 allele, two pathogenic sequence variants on opposite SMN1 alleles, and one pathogenic sequence variant and the loss of exon 8 on the opposite SMN1 allele.

Mitochondrial variants are detected with this test. Single nucleotide variants (SNVs) down to a 1% heteroplasmy level can be detected. Large deletions and duplications of mitochondrial DNA are also validated to be detected by this test. Interpretation of the mitochondrial genome is performed as part of this test.

Incidental findings may be reported if the patient and/or patient's family do not opt-out of receiving these results. RCIGM's internal incidental finding policy includes the following: 1) variant must be classified as pathogenic per ACMG guidelines and be in alignment with the known inheritance pattern of the genetic condition; 2) the variant is located in a gene with a well established gene-disease relationship; 3) the gene and associated condition are shown to be medically actionable as established by RCIGM policy and in consultation with the RCIGM clinical team. Pathogenic variants included within the ACMG list of 59 secondary findings genes that are identified will be reported, as well as other findings that meet the above criteria.

Assay Limitations

Full coverage of the genome is not currently possible due to technically challenging repetitive elements and duplicated regions within the genome. Thus, not all regions of the genome are sequenced and/or uniquely aligned to the reference genome. Certain genomic alterations may not be detected with the current version of this test. For example, genomic alterations such as trinucleotide repeat expansions and translocations will not be identified with the current version of the test. This test is set up to evaluate the potential contribution of rare disease causing variants in known disease genes. It is not designed to evaluate for common variants in genes that might contribute to disease risk nor for disorders that have a multi-genic inheritance. Based on current knowledge, potential disease causing variants may not always be recognized at the time of testing.

Regulatory Disclosures

This test was developed and its performance characteristics determined by the Rady Children's Hospital and the Rady Children's Institute for Genomic Medicine. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. RCIGM has established and verified the test's accuracy and precision as outlined in the requirements of CLIA '88. The test is used for clinical purposes. It should not be regarded as investigational or for research. The Rady Children's Institute for Genomic Medicine is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.