STAT Whole Genome Sequencing TRIO with Parental Reports, plus StepOne® Biochemical Profile

Test Code	D2321
Test Summary	STAT Diagnostic whole genome sequencing and mitochondrial genome sequencing of the proband and 2 family members as well as a biochemical screen of >50 inhertited disorders in the proband. Additional healthy analysis and interpretation performed on the family members.
Turn-Around-Time (TAT)*	10 - 12 days
Acceptable Sample Types	Dried Blood Spots (Preferred sample type) Whole Blood (EDTA)
Acceptable Billing Types	Self (patient) Payment Institutional Billing

Indications for Testing

- Genetically heterogeneous disease caused by likely pathogenic/pathogenic findings in multiple genes
- Condition suggestive of a genetic disorder with a long differential diagnosis list
- Unclear or atypical presentation of a genetic disorder
- Previous genetic testing did not yield a diagnosis, including exome sequencing

Test Description

This test involves sequencing of the whole genome with mean coverage of 40X and complete coverage of over 5,400 disease-associated genes. All variants identified will be analyzed according to American College of Medical Genetics and Genomics (ACMG) guidelines. In addition to SNVs, our WGS test will reliably detect CNVs of 3 exons or greater as well as large-scale CNVs such as microdeletions and other gene/chromosomal-level events. CNVs of 1-2 exons may be detected and reported with the recommendation for follow-up testing. Mitochondrial DNA analysis is included. In addition to primary analysis, patients can opt-in to a comprehensive secondary analysis including the recommended list by ACMG. Family samples are tested concurrently with the proband sample to further elucidate potential pathogenic changes. Parental reports with secondary findings are also generated. Please note, appropriate selection must be made on the test requisition form as well as the consent in order to generate parental reports. Additionally, a comprehensive biochemical profile is performed to identify the presence of more than 50 inherited disorders, the full core and secondary panel recommended by the American College of Medical Genetics. This includes conditions that may not be included in state-mandated programs.

Test Methods and Limitations

Whole genome sequencing is performed on genomic DNA using 2X150bp reads on Illumina next generation sequencing (NGS) systems at a mean coverage of 40X in the target region. The target region includes coding exons and 10bp of flanking intronic sequence of the known protein coding RefSeq genes. This sequencing provides >97% coverage of the 22,000 genes in the genome at >40x. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. A list of low coverage regions is available upon request. Alignment to the human reference genome (hg19) is performed and annotated variants are identified in the targeted region. Variants are called at a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Single nucleotide variants (SNVs) meeting internal quality assessment guidelines are confirmed by Sanger sequence analysis for records after results are reported. Indels and SNVs may be confirmed by Sanger sequence analysis before reporting at director discretion. This assay cannot detect variants in areas containing large numbers of tandem repeats. Mitochondrial DNA is sequenced and analyzed using the same pipeline. Copy number variation (CNV) analysis is designed to detect deletions and duplications of three exons or more; in some instances, due to the size of the exons or other factors, not all CNVs may be analyzed. Only CNVs related to phenotype are reported. This assay is not designed to detect mosaicism; possible cases of mosaicism may be investigated at the discretion of the laboratory director. Primary data analysis is performed using Illumina DRAGEN Bio-IT Platform v.2.03. Secondary and tertiary data analysis is performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.4.3 or Illumina DRAGEN Bio-IT Platform v.2.03 for CNV and absence of heterozygosity (AOH). The StepOne Comprehensive Biochemical

Profile is performed by tandem mass spectrometry as well as other select technologies.

Detailed Sample Requirements

Dried Blood Spots (Preferred sample type)

Collection Container(s):

Dried blood spot card

Collection:

Follow kit instructions. Briefly, allow blood to saturate card until indicated areas are filled and blood has soaked through card. Air dry card at ambient temperature for at least 3 hours.

- **NBS:** Please contact PKIG to request the StepOne® kit.
- **Gene Sequencing:** Please contact PKIG to request the DBS collection kit.
- **For pre-punched DBS:** The required minimum 6 punches with 3.2 mm or 4 punches 4.75 mm.
  Sample Condition: Follow the instructions provided with the collection set. Store the dried blood at ambient temperature for up to two days. If the specimen cannot be sent as soon as it is dry, the filter paper should be placed in a sealable plastic bag and stored in a refrigerator (? 8°C) or preferably in a freezer.
  Shipping: Follow kit instructions. Double bag and ship overnight at ambient temperature.

Whole Blood (EDTA)

Collection Container(s):

EDTA (purple top)

Collection:

Infants (< 2-years): 2 to 3 mL; Children (>2-years): 3 to 5 mL; Older children and adults: Minimum 5mL. The blood tube should be inverted several times immediately after blood collection to prevent coagulation.

Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature ensuring receipt within 5-days of collection.

SPECIAL INSTRUCTIONS: Clotted or hemolyzed samples are not accepted.