STAT Prenatal Whole Exome Sequencing, TRIO

Test Code	D1310E
Test Summary	STAT prenatal whole exome sequencing (WES)
Turn-Around-Time (TAT)*	7 - 10 days
Acceptable Sample Types	Cultured Amniocytes Cultured Chorionic Villi DNA, Isolated Products of Conception
Acceptable Billing Types	Self (patient) Payment Institutional Billing Commercial Insurance

Indications for Testing

Increased NT/cystic hygroma
Fetal anomaly

Test Description

This STAT test is for prenatal use and involves sequencing the whole exome with a mean coverage of 100x, enhanced coverage of known disease-causing genes, and curated deep-intronic variants. The WES test will reliably detect the majority of copy number variations (CNVs) of 3 exons or greater. Smaller CNV events may also be detected and reported, but additional follow-up testing is recommended if a smaller CNV is suspected. The WES assay will also detect microdeletion/duplication events greater than 500kb in clinically relevant regions, although follow-up testing may be warranted to better delineate the exact size of the event and confirm breakpoints. Secondary findings per American College of Medical Genetics (ACMG) recommendations may be requested; however, secondary findings will not be reported for ongoing pregnancies. Proband only, duo, trio and quad testing options are available, with parental reports included on duo, trio and quad testing if specifically requested.

Condition Description

The purpose of this test is to find the underlying genetic cause for prenatal findings.

Test Methods and Limitations

Whole exome sequencing is performed on genomic DNA using the Agilent SureSelect Clinical Research Exome v3 targeted sequence capture method to enrich for the exome. Direct sequencing of the amplified captured regions was performed using 2X150bp reads on Illumina next generation sequencing (NGS) systems. 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, if any, is available upon request. Alignment to the human reference genome (hg19) is performed and annotated variants are identified in the targeted region. Variants reviewed have a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Indels and single nucleotide variants (SNVs) may be confirmed by Sanger sequence analysis before reporting at director discretion. Mitochondrial DNA is sequenced and analyzed using the same pipeline. Mitochondrial variants are reported at a minimum of 5% heteroplasmy if the average coverage of the mitochondrial genome is 1000x. This assay cannot detect variants in regions of the exome that are not covered, such as deep intronic, promoter, and enhancer regions, and areas containing large numbers of tandem repeats. Genes and/or exons located in pseudogene regions are not covered in this assay. 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. This assay does not interrogate CNVs in mitochondrial DNA. CNV analysis will not detect tandem repeats, balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and balanced insertions), methylation abnormalities, triploidy, and genomic imbalances in segmentally duplicated regions. 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 bcl2fastq converter v2.19. Secondary

analysis is performed using Illumina DRAGEN Bio-IT Platform v.3.4.12. Tertiary data analysis is performed using SnpEff v4.3t and PerkinElmer's internal ODIN v.1.01 software. CNV and absence of heterozygosity are assessed using BioDiscovery's NxClinical v6.1 software.

Detailed Sample Requirements

Cultured Amniocytes

SPECIAL INSTRUCTIONS: Please contact a PKIG Laboratory genetic counselor for these requests.

Cultured Chorionic Villi

Collection Container(s):

Two T-25 flasks

Collection:

All prenatal specimens will be tested for maternal cell contamination (MCC). Send maternal blood (EDTA tube) for comparison. If blood is unavailable, we will accept genomic DNA and Saliva sample types.

Sample Condition: Transfer cultured amniocytes or cultured CVS to two T-25 flasks at ?80% confluence.
Shipping: Cultures topped off with sterile medium and shipped immediately at ambient temperature by overnight express with arrival Monday-Friday only.

SPECIAL INSTRUCTIONS: For prenatal samples, PerkinElmer will provide a shipping label to use for shipping the sample to our lab. Please call 1 (866) 354-2910 to arrange this. At this time, you will also be connected to a laboratory genetic counselor to answer any questions about the testing.

DNA, Isolated

Collection:

Required DNA Quantity by Test Type*:

- **Next Generation Sequencing (NGS):** Send >1000 ng total gDNA @ >15 ng/?L. Please ship samples in 10mM Tris. Do not use EDTA.
- **Sanger Sequencing:** Send >500 ng total gDNA @ >15 ng/?L (varies by the size of the gene and the variants requested).
- **Non-Sanger Sequencing Tests:** Send >500 ng total gDNA @ >15 ng/?L.

Sample Condition: * Required DNA Quality: High molecular weight DNA (>12kb). A260/A280 reading should be ? 1.8. A260/230 a ratio range of 1.8 to 2.2. Contact the laboratory for specific amounts if total ng cannot be met.
Shipping: Ship overnight at ambient temperature.

SPECIAL INSTRUCTIONS:

- **Research Laboratories:** DNA extracted in research laboratories is not acceptable. Only under exceptional circumstances (e.g., proband not available) will DNA extracted in a research laboratory be accepted for clinical testing. Additional testing (e.g., of other family members) may be required to confirm results.
- **Laboratories outside the United States:** Non-US laboratories are not subject to CLIA regulations and will be reviewed on a case-by-case basis. Please call to speak with a laboratory genetic counselor prior to submitting a DNA sample from any non-CLIA certified laboratory.
- **Special Notes**: If extracted DNA is submitted, information regarding the method used for extraction should be sent along with the sample.

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