Expanded Newborn Screening and Gene Sequencing Panel with *ABCD1* and *CYP21A2*

Test Code	D3004
Test Summary	This panel analyzes 269 genes that have been associated with conditions manifesting in the newborn and pediatric periods of life.
Turn-Around-Time (TAT)*	3 - 5 weeks
Acceptable Sample Types	Whole Blood (EDTA) (Preferred sample type) DNA, Isolated Dried Blood Spots Saliva
Acceptable Billing Types	Self (patient) Payment Institutional Billing Commercial Insurance

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

Test Description

This test analyzes 269 genes that have been associated with conditions manifesting in the newborn and pediatric periods of life. Both sequencing and deletion/duplication (CNV) analysis will be performed on the coding regions of all genes included (unless otherwise marked). All analysis is performed utilizing Next Generation Sequencing (NGS) technology. CNV analysis detects most deletions and duplications of three exons or greater in size. Smaller CNV events may also be detected and reported, but additional follow-up testing is recommended if a smaller CNV is suspected. All variants are classified according to ACMG guidelines.

Genes

AASS, ABCC8, ABCD1, ABCD4, ABCG5, ABCG8, ACAD8, ACAD9, ACADM, ACADS, ACADSB, ACADVL, ACAT1, ACSF3, ADA, ADK, AGA, AGL, AGPAT2, AGXT, AHCY, AK2, ALDH4A1, ALDH5A1, ALDOB, AMT, ARG1, ARSA, ARSB, ASL, ASS1, ATM, ATP2B2, ATP5E, ATP7A, ATP7B, AUH, BCKDHA, BCKDHB, BSCL2, BTD, CAV1, CBS, CD247, CD320, CD3D, CD3E, CD3G, CD3Z, CDH23, CFC1, CFTR, CIITA, CLN3, CLN5, CLN6, CLN8, CLRN1, CORO1A, CPS1, CPT1A, CPT2, CRYGD, CTH, CTNS, CYP11B1, CYP17A1, CYP21A2, CYP27A1, DBT, DCLRE1C, DECR1, DLD, DNAJC19, DOCK8, DPYD, DUOX2, DUOXA2, ENO3, EPM2A, ETFA, ETFB, ETFDH, ETHE1, EYA1, F5, FAH, FBP1, FOXH1, FOXI1, FOXN1, FTCD, G6PC, G6PD, GAA, GALC, GALE, GALK1, GALNS, GALT, GATA4, GATA6, GBE1, GCDH, GCH1, GCK, GCSH, GDF1, GJA1, GLA, GLB1, GLDC, GM2A, GNE, GNMT, GNPTAB, GNS, GPR98, GRHPR, GSS, GUSB, GYS1, GYS2, HADH, HADHA, HADHB, HAL, HAND1, HBA1, HBA2, HBB, HELB, HGD, HGSNAT, HLCS, HMGCL, HMGCS2, HPD, HSD17B10, HSD17B3, HSD3B2, HYAL1, IDS, IDUA, IL2RG, IL7R, INS, IVD, IYD, JAK3, KCNJ10, KCNJ11, LAMP2, LIG4, LIPA, LMBRD1, LPL, MAN2B1, MANBA, MAT1A, MCCC1, MCCC2, MCEE, MCOLN1, MED13L, MFSD8, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MTR, MTRR, MUT, MYO7A, NADKD1, NAGA, NAGLU, NAGS, NEU1, NHEJ1, NHLRC1, NKX2-5, NKX2-6, NOTCH1, NPC1, NPC2, OAT, OPA3, OPLAH, ORAI1, OTC, PAH, PAX8, PC, PCBD1, PCCA, PCCB, PCDH15, PDHA1, PDHX, PDP1, PDX1, PFKM, PGAM2, PGM1, PHKA1, PHKA2, PHKB, PHKG2, PMM2, PNP, POLG, PRKAG2, PRKDC, PRNP, PRODH, PSAP, PTPRC, PTRF, PTS, PYGL, PYGM, QDPR, RAC2, RAG1, RAG2, RFX5, RFXANK, RFXAP, RMRP, SC5D, SERAC1, SERPINA1, SGSH, SIX1, SIX5, SLC17A5, SLC22A5, SLC25A13, SLC25A15, SLC25A20, SLC2A2, SLC37A3, SLC37A4, SLC5A5, SLC7A7, SMAD6, SMPD1, SOAT1, SPR, SRD5A2, STAT5B, STIM1, SUCLA2, SUCLG1, SUMF1, TAT, TAZ, TBX1, TG, TH, THRA, TMEM70, TPO, TPP1, TRHR, TSHB, TSHR, USH1C, USH1G, USH2A, VPS13A, ZAP70, ZFPM2, ABCD1, CYP21A2, AASS, ABCC8, ABCD1, ABCD4, ABCG5, ABCG8, ACAD8, ACAD9, ACADM, ACADS, ACADSB, ACADVL, ACAT1, ACSF3, ADA, ADK, AGA, AGL, AGPAT2, AGXT, AHCY, AK2, ALDH4A1, ALDH5A1, ALDOB, AMT, ARG1, ARSA, ARSB, ASL,

ASS1, ATM, ATP2B2, ATP5E, ATP7A, ATP7B, AUH, BCKDHA, BCKDHB, BSCL2, BTD, CAV1, CBS, CD247, CD320, CD3D, CD3E, CD3G, CD3Z, CDH23, CFC1, CFTR, CIITA, CLN3, CLN5, CLN6, CLN8, CLRN1, CORO1A, CPS1, CPT1A, CPT2, CRYGD, CTH, CTNS, CYP11B1, CYP17A1, CYP21A2, CYP27A1, DBT, DCLRE1C, DECR1, DLD, DNAJC19, DOCK8, DPYD, DUOX2, DUOXA2, ENO3, EPM2A, ETFA, ETFB, ETFDH, ETHE1, EYA1, F5, FAH, FBP1, FOXH1, FOXI1, FOXN1, FTCD, G6PC, G6PD, GAA, GALC, GALE, GALK1, GALNS, GALT, GATA4, GATA6, GBE1, GCDH, GCH1, GCK, GCSH, GDF1, GJA1, GLA, GLB1, GLDC, GM2A, GNE, GNMT, GNPTAB, GNS, GPR98, GRHPR, GSS, GUSB, GYS1, GYS2, HADH, HADHA, HADHB, HAL, HAND1, HBA1, HBA2, HBB, HGD, HGSNAT, HLCS, HMGCL, HMGCS2, HPD, HSD17B10, HSD17B3, HSD3B2, HYAL1, IDS, IDUA, IL2RG, IL7R, INS, IVD, IYD, JAK3, KCNJ10, KCNJ11, LAMP2, LIG4, LIPA, LMBRD1, LPL, MAN2B1, MANBA, MAT1A, MCCC1, MCCC2, MCEE, MCOLN1, MED13L, MFSD8, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MTR, MTRR, MUT, MYO7A, NADKD1, NAGA, NAGLU, NAGS, NEU1, NHEJ1, NHLRC1, NKX2-5, NKX2-6, NOTCH1, NPC1, NPC2, OAT, OPA3, OPLAH, ORAI1, OTC, PAH, PAX8, PC, PCBD1, PCCA, PCCB, PCDH15, PDHA1, PDHX, PDP1, PDX1, PFKM, PGAM2, PGM1, PHKA1, PHKA2, PHKB, PHKG2, PMM2, PNP, POLG, PRKAG2, PRKDC, PRNP, PRODH, PSAP, PTPRC, PTRF, PTS, PYGL, PYGM, QDPR, RAC2, RAG1, RAG2, RFX5, RFXANK, RFXAP, SC5D, SERAC1, SERPINA1, SGSH, SIX1, SIX5, SLC17A5, SLC22A5, SLC25A13, SLC25A15, SLC25A20, SLC2A2, SLC37A3, SLC37A4, SLC5A5, SLC7A7, SMAD6, SMPD1, SPR, SRD5A2, STAT5B, STIM1, SUCLA2, SUCLG1, SUMF1, TAT, TAZ, TBX1, TG, TH, THRA, TMEM70, TPO, TPP1, TRHR, TSHB, TSHR, USH1C, USH1G, USH2A, VPS13A, ZAP70, ZFPM2, AASS, ABCC8, ABCD1, ABCD4, ABCG5, ABCG8, ACAD8, ACAD9, ACADM, ACADS, ACADSB, ACADVL, ACAT1, ACSF3, ADA, ADK, AGA, AGL, AGPAT2, AGXT, AHCY, AK2, ALDH4A1, ALDH5A1, ALDOB, AMT, ARG1, ARSA, ARSB, ASL, ASS1, ATM, ATP2B2, ATP5E, ATP7A, ATP7B, AUH, BCKDHA, BCKDHB, BSCL2, BTD, CAV1, CBS, CD247, CD320, CD3D, CD3E, CD3G, CD3Z, CDH23, CFC1, CFTR, CIITA, CLN3, CLN5, CLN6, CLN8, CLRN1, CORO1A, CPS1, CPT1A, CPT2, CRYGD, CTH, CTNS, CYP11B1, CYP17A1, CYP21A2, CYP27A1, DBT, DCLRE1C, DECR1, DLD, DNAJC19, DOCK8, DPYD, DUOX2, DUOXA2, ENO3, EPM2A, ETFA, ETFB, ETFDH, ETHE1, EYA1, F5, FAH, FBP1, FOXH1, FOXI1, FOXN1, FTCD, G6PC, G6PD, GAA, GALC, GALE, GALK1, GALNS, GALT, GATA4, GATA6, GBE1, GCDH, GCH1, GCK, GCSH, GDF1, GJA1, GLA, GLB1, GLDC, GM2A, GNE, GNMT, GNPTAB, GNS, GPR98, GRHPR, GSS, GUSB, GYS1, GYS2, HADH, HADHA, HADHB, HAL, HAND1, HBA1, HBA2, HBB, HGD, HGSNAT, HLCS, HMGCL, HMGCS2, HPD, HSD17B10, HSD17B3, HSD3B2, HYAL1, IDS, IDUA, IL2RG, IL7R, INS, IVD, IYD, JAK3, KCNJ10, KCNJ11, LAMP2, LIG4, LIPA, LMBRD1, LPL, MAN2B1, MANBA, MAT1A, MCCC1, MCCC2, MCEE, MCOLN1, MED13L, MFSD8, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MTR, MTRR, MUT, MYO7A, NADKD1, NAGA, NAGLU, NAGS, NEU1, NHEJ1, NHLRC1, NKX2-5, NKX2-6, NOTCH1, NPC1, NPC2, OAT, OPA3, OPLAH, ORAI1, OTC, PAH, PAX8, PC, PCBD1, PCCA, PCCB, PCDH15, PDHA1, PDHX, PDP1, PDX1, PFKM, PGAM2, PGM1, PHKA1, PHKA2, PHKB, PHKG2, PMM2, PNP, POLG, PRKAG2, PRKDC, PRNP, 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SLC5A5, SLC7A7, SMAD6, SMPD1, SPR, SRD5A2, STAT5B, STIM1, SUCLA2, SUCLG1, SUMF1, TAT, TAZ, TBX1, TG, TH, THRA, TMEM70, TPO, TPP1, TRHR, TSHB, TSHR, USH1C, USH1G, USH2A, VPS13A, ZAP70, ZFPM2

Test Methods and Limitations

Sequencing is performed on genomic DNA using an Agilent targeted sequence capture method to enrich for the genes on this panel. Direct sequencing of the amplified captured regions was performed using 2X100bp 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. Low coverage regions, if any, are limited to ~1% or less of the nucleotides in the test unless a pathogenic variant explaining the phenotype is discovered. A list of these 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 are confirmed by Sanger sequence analysis before reporting at the director's discretion. This assay cannot detect variants in regions of the exome that are not covered, such as deep intronic, promoter, and enhancer regions, areas containing large numbers of tandem repeats, and variants in mitochondrial DNA. 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 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.3.4.12. Secondary and tertiary data analysis is performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.6.1 or Illumina DRAGEN Bio-IT Platform v.3.4.12 for CNV and absence of heterozygosity (AOH). Genes and/or exons located in pseudogene regions are not covered in this assay.

Detailed Sample Requirements

Whole Blood (EDTA) (Preferred sample type)

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.

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.

Dried Blood Spots

Saliva

Collection Container(s):

Oragene™ Saliva Collection Kit or ORAcollect-Dx kit

Collection:

Collect saliva on an Oragene™ Saliva Collection Kit ORAcollect-Dx kit according to the manufacturer's instructions.

Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature.

SPECIAL INSTRUCTIONS: Please contact PerkinElmer to request the saliva collection kit for patients that cannot provide a blood sample as whole blood is the preferred sample.