Lab Dept: Anatomic Pathology

Test Name: ANIRIDIA (PAX6) SEQUENCING & DELETION /DUPLICATION

General Information

Lab Order Codes: PAX

Synonyms: Developmental Eye Disorders Gene Testing; WT1 Sequencing; DCDC1 Sequencing; ELP4 Sequencing

CPT Codes: 81479 – Unlisted Molecular Pathology procedure (PAX6 del/dup)

Test Includes: Using genomic DNA obtained from an EDTA blood sample, bi-directional sequence of the coding and non-coding exons of the PAX6 gene (exons 1-13), the alternatively spliced exon 5a and splice junctions are obtained and analyzed. Deletion/duplication testing by targeted array CGH analysis with exon-level resolution (EXONArrayDx) is performed concurrently to evaluate for a deletion or duplication of one or more exons of the PAX6, DCDC1m ELP4 and WT1 genes. Mutations are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method.

Logistics

Test Indications: Aniridia is a developmental anomaly of the entire eye, characterized by varying degrees of iris hypoplasia. Ocular abnormalities associated with aniridia include persistent papillary membrane, congenital cataracts, ectopia lentis, developmental glaucoma, corneal pannus with progressive keratophathy and foveal hypoplasia. The most severe presentation of aniridia is the complete absence of the iris. Milder disease may include enlargement and irregularity of the pupil and small, slit-like defects in the anterior layer. Vision is variably affected, and the severity of vision loss tends to correlate with the presence of other associated ocular defects. Approximately 70% of the cases with isolated aniridia (ie, aniridia without associated anomalies) are familial while the remaining 30% of cases are sporadic. Aniridia may be caused by heterozygous mutations in the PAX6 gene. PAX6 mutations have also been described in a host of other ocular developmental abnormalities that appear clinically distinct from aniridia, including: microphthalmia with or without colobama; optic nerve hypoplasia and other congenital optic nerve anomalies; and a specific form of corneal dystrophy. Aniridia may also be seen as part of the WAGR (Wilm's tumor, aniridia, genital anomalies and mental retardation) syndrome, which is caused by a deletion of chromosome 11p13, the genomic region harboring both PAX6 and WT1 genes. Some cases of sporadic aniridia involve de novo submicroscopic deletions in this chromosomal region and therefore could place the patient at risk for developing Wilm's tumor.

Sequence changes in the PAX6 gene associated with aniridia are nearly always truncating mutations leading to haploinsufficiency. Other

	developmental eye anomalies, such as congenital nystagmus, ectopia, keratopathy, and juvenile cataracts, have been associated with missense mutation in the PAX6 gene. Deletion of the PAX6 gene is known to cause aniridia, and contiguous gene deletions that include the WT1 gene are associated with WAGR syndrome. **Reasons for referral:** 1. Confirmation of clinical diagnosis 2. Determination of the molecular basis of aniridia in patients at risk for Wilm's tumor 3. Genetic counseling 4. Prenatal diagnosis
Lab Testing Sections:	Anatomic Pathology - Sendouts
Referred to:	GeneDx, Inc. (GDX#: 491)
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Daily, 24 hours (Preferred draws are Monday – Friday as specimens can only be received at the reference lab Monday - Saturday. Specimens collected Saturday or Sunday will be held for shipment on Monday.)
Turnaround Time:	3 weeks
Special Instructions:	A GeneDx signed request form must be sent with any patient or specimen to the laboratory.

Specimen

Specimen Type:	Whole blood
Container:	Lavender top (EDTA) tube
Draw Volume:	1 - 5 mL blood
Processed Volume:	Same as Draw Volume
Collection:	Routine venipuncture for blood specimens, invert gently to mix
Special Processing:	Lab Staff: Send whole blood in original collection container labeled with patient name, date of birth and medical record number, including signed consent form and requisition, with a cool pack, via overnight or second-day courier so that the sample will arrive at GeneDx Monday through Saturday. Samples drawn on Saturday or Sunday should be held at refrigerated temperatures for shipment on Monday. **Do not** freeze. **Note:** Specimens may be stored at refrigerated temperatures for up to 7 days prior to shipping.

Patient Preparation:	None
Sample Rejection:	Unrefrigerated specimens older than 48 hours; clotted or hemolyzed for blood; frozen specimens; mislabeled or unlabeled specimens

Interpretive

Reference Range:	No mutations detected
Critical Values:	N/A
Limitations:	In cases of aniridia without a detectable PAX6 gene deletion, over 80% of individuals were found to have a small intragenic mutation in the PAX6 gene, with a higher proportion in familial cases than in sporadic cases. One study also showed that 2 of 18 cases (11%) with other eye abnormalities (outside the aniridia spectrum) had a missense mutation in the PAX6 gene. In a study of 70 unrelated probands affected with aniridia, deletions of one or more exons of the PAX6 gene itself was intact (Redker EJ et al, 2008). The combination of sequencing and deletion testing has a sensitivity of approximately 90% for the identification of a mutation in a patient diagnosed with aniridia.
Methodology:	Large gross chromosomal deletion can be detected by cytogenic analysis, fluorescent in situ hybridization (FISH) and oligo array Comparative Genomic Hybridization (oligoACGH) analysis. However, these methods will not be detect partial PaX6 gene deletions involving only one or a few exons. Deletion/Duplication testing by targeted array CGH analysis with exon-level resolution (ExonArrayDx) is performed to evaluate for a deletion or duplication of one or more exons. While heterozygous PAX6 mutations were also found in a few patients with syndromic anaophthalmia.
References:	GeneDx, Inc. January 2018 (301) 519-2100 Fax (301) 519-2892