

The results of these tests should be interpreted and utilized after review of the following specifications:

Indications

These tests are indicated for individuals with suspected Familial Long QT Syndrome (LQTS), Brugada Syndrome (BrS), Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), Dilated Cardiomyopathy (DCM), Hypertrophic Cardiomyopathy (HCM), and related syndromes, or for family members of individuals who have tested positive for a genetic variant associated with one of these conditions. These tests identify genetic variants in genes in which mutations have been shown to cause or create susceptibility to these syndromes.

Description of Genetic Assays

The *FAMILION* tests include 7 testing options. The first 6 options are index tests, which include comprehensive sequence determination and variant detection in open reading frame and intronic sequences containing splice junction sites for the included exons, while the last option is intended for family members of a person in whom one or more mutations was detected.

- 1. LQTS Test.** Provides analysis of 11 genes (94 amplicons) associated with LQTS. All coding exons of the following genes are analyzed: *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), *KCNE2* (LQT6), *KCNJ2* (LQT7), *CAV3* (LQT9), *SCN4B* (LQT10), and *SNTA1* (LQT12). Additionally, exons 8 and 9 of *CACNA1C* (LQT8) and exon 18 of *AKAP9* (LQT11), which encompass all LQTS-associated mutations reported in these genes as of April, 2009, are analyzed. Seven specific Andersen-Tawil syndrome (ATS) associated mutations in *KCNJ2* are not analyzed: NM_000891.2:c.212A>T, 407C>T, 430G>A, 652C>T, 653G>A, 899G>T, 907G>A.
- 2. BrS Test.** Provides analysis covering all coding exons of the gene *SCN5A* (34 amplicons).
- 3. CPVT Test.** Provides analysis covering all or selected coding exons of 2 genes (72 amplicons) associated with CPVT. The following 66 *RYR2* exons are analyzed, which includes all those in which CPVT-associated mutations have been reported as of April, 2009, and others that are adjacent: 1-28, 37-50, 75, 83-105. The entire coding region of *KCNJ2* is analyzed; however, seven specific ATS-associated mutations in *KCNJ2* are not analyzed: NM_000891.2:c.212A>T, 407C>T, 430G>A, 652C>T, 653G>A, 899G>T, 907G>A.
- 4. ARVC Test.** Provides analysis covering all coding exons of 5 genes (96 amplicons) associated with ARVC as follows: *DSC2*, *DSG2*, *DSP*, *PKP2*, and *TMEM43*.
- 5. DCM Test.** Provides analysis covering all coding exons of 12 genes (173 amplicons) associated with DCM as follows: *ANKRD1*, *ACTC1*, *LDB3*, *LMNA*, *MYBPC3*, *MYH7*, *PLN*, *SCN5A*, *TNNC1*, *TNNI3*, *TNNT2* and *TPM1*.
- 6. HCM Test.** Provides analysis covering all coding exons of 12 genes (146 amplicons) associated with HCM as follows: *ACTC*, *GLA*, *LAMP2*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *PRKAG2*, *TNNT2*, *TNNI3*, *TNNC1*, and *TPM1*.

7. Family Specific Test. Provides analysis of one or more Class I or II variants (see below) found in an index case of one of the tests described above and is indicated for testing blood relatives of the index case. Includes sequence determination and variant detection in the gene region(s) in which mutation(s) were detected in the index case for the family. If additional mutations or variants are found in the Family Specific Test, these will also be reported.

Description of Methods

- 1. Sample acquisition:** An 8 ml blood sample in two (2) 4 ml EDTA tubes provided in the sample collection kit is required and shipped overnight at room temperature to the test laboratory. Genomic DNA is isolated from fresh whole blood. DNA, frozen blood, and other tissue samples are also accepted if they meet specific criteria; please contact our Customer Service Department for details.
- 2. DNA Sequence Analysis:** DNA amplification by polymerase chain reaction (PCR) is used to generate templates for direct sequencing. A number of PCR amplicons are utilized to obtain coverage of the complete open reading frame, splice junction sites and flanking regions for the targeted regions of each gene. Directed sequencing is performed in both forward and reverse directions using dye-terminator chemistries for all regions except for those regions that, due to constraints within the particular DNA sequence, are amplified twice and sequenced in a single direction. Automated electrophoretic separation of sequencing reactions is performed.
- 3. Variant Detection:** Sequence traces are analyzed for heterozygous or homozygous variants with respect to public reference sequences that have been confirmed by sequencing hundreds of individuals of diverse ancestry. Sequence traces are computationally and visually compared with reference traces to identify and validate variant calls. In order to be analyzed, each trace must meet rigorous quality standards. Two technologists independently score all traces for variants and a supervisor reconciles discrepancies.
- 4. Report Generation:** Class I, II, and III variants, as defined below, are reported. The final report is reviewed and signed by a CLIA licensed Laboratory Director.

Performance Characteristics

- 1. Analytical Specificity (Index Tests):** The chance of a falsely detected genetic variant is minimized by requiring that each variant be seen in independent sequence traces and that two trained technologists independently examine each trace. For each positive finding of a Class I or Class II variant (see definitions below), a second round of PCR amplification and sequencing is performed to confirm the initial finding. Chances of false positives are further minimized by using a validated sample tracking system involving robotics and barcodes.
- 2. Analytical Sensitivity (Index Tests):** Failure to detect a variant in an analyzed amplicon could be due to an amplicon being refractory to analysis by direct DNA sequencing, sample mishandling, sample tracking errors or errors in data analysis. The rate of such errors is estimated to be < 1%.
- 3. Clinical Sensitivity:** It is estimated that detectable variants in these gene panels respectively account for 75-80% of LQTS,

15-25% of BrS, 65-75% of CPVT, 40-50% of ARVC, 25% of DCM, and 50-60% of HCM (Tester et al. *Heart Rhythm* 2:507-17, 2005; Napolitano and Priori. *Heart Rhythm* 4:675-78, 2007; Ruan et al. *Circulation*. 116:II_492, 2007; Taggart et al. *Circulation* 115:2613-20, 2007; Sen-Chowdhry et al. *J Am Coll Cardiol* 50:1813-21, 2007; Keren et al. *Nat Clin Pract Cardiovasc Med* 5:158-68, 2008; Medeiros-Domingo et al. *Heart Rhythm*. 2009;6:S102; Hayashi et al. *Circulation* 119:2426-34, 2009; Data on file, PGxHealth).

4. Clinical Specificity: Variants that would have been called possible or probable deleterious if seen in a patient have been found in apparently unaffected individuals in the genes included in the tests for LQTS, BrS, CPVT, HCM and ARVC. Therefore, comprehensive clinical evaluation is strongly recommended to direct treatment decisions.

5. Family Specific Test: The analytical sensitivity and specificity of these analyses are approximately 100%.

6. Limitations: There may be amplicons for which it is not possible to generate traces in both directions. Rare polymorphisms may exist that could lead to false negative or false positive results. These tests will not detect large DNA rearrangements or deletions and will not detect errors in RNA transcription or processing that are unrelated to coding sequence variants of DNA exons. The tests will not detect mutations in non-targeted exons. For many of these syndromes, mutations in other genes have been implicated as rarely causative and are not tested in these analyses.

Variant Classification and Interpretation

DNA variants are identified and classified by comparison with reference sequences and the PGxHealth Variant Database. This database is produced through review of published literature and through results of PGxHealth's sequencing. This database contains an extensive collection of common polymorphisms and rare variants in these genes that are not expected to confer susceptibility to congenital cardiac syndromes; these variants were found in comprehensive scanning of the genes in several hundred individuals of diverse race and ethnicity and from study of the literature, together referred to as the "Reference Panel", which differs in composition across the tested genes. The healthy individuals in the Reference Panel were not known to have inherited cardiac syndromes (Ackerman et al. *Mayo Clin Proc* 78:1479-87, 2003; Ackerman et al. *Heart Rhythm* 1:600-7, 2004). Expert scientists ensure variant classification and interpretation reflect current, published information.

Each variant that is detected is categorized into one of 4 classes. These classes and classification guidelines are described below. Based on additional evidence, exceptions to these guidelines are made. *Note:* These classes should not be confused with the designations such as LQT1, LQT2, LQT3, etc., which relate to the syndrome and affected gene.

CLASS I: Deleterious and Probable Deleterious Mutations *KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*

1. Evidence of deleteriousness
2. Nonsense variant
3. Missense single nucleotide variant not seen in the Reference Panel in transmembrane-spanning domain or pore
4. Insertion or deletion
 - a. Frameshift variant

- b. In-frame variant in transmembrane-spanning domain or pore

5. Canonical splice site variant

All Other Genes

1. Evidence of deleteriousness
2. Nonsense variant
3. Insertion or deletion causing a frameshift
4. Canonical splice site variant

CLASS II: Possible Deleterious Mutations (Variants of Uncertain Significance)

KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2

1. Missense single nucleotide variant not seen in the Reference Panel and not in transmembrane-spanning domain or pore
2. Rare missense single nucleotide variant seen in the Reference Panel, but with published evidence of deleteriousness
3. In-frame insertion or deletion not in transmembrane-spanning domain or pore
4. Non-canonical splice site variant

All Other Genes

1. Missense single nucleotide variant not seen in the Reference Panel
2. Rare Missense single nucleotide variant seen in the Reference Panel, but with published evidence of deleteriousness
3. In-frame insertion or deletion
4. Non-canonical splice site variant

CLASS III: Polymorphisms (Variants Not Generally Expected to Cause Disease)

1. Predicted protein-altering variant seen in the Reference Panel with either
 - a. Common frequency or
 - b. Rare frequency and without published evidence of deleteriousness

CLASS IV: Non-Protein-Altering Variants

All non-coding and synonymous variants (no changes in encoded amino acid) except those predicted to affect intron splicing, which are categorized as Class II Mutations (splice variants). These variants do not alter the protein coding sequence. Because of the lack of known or suspected clinical significance, these variants are not reported, however are available upon request.

Recommendation for family member testing: In cases where a Class I or Class II mutation is found, a recommendation for clinical evaluation and possible genetic testing of first-degree blood relatives will be included in the report.

Change of interpretation and amended reports: If there is a change in the clinical interpretation of a reported variant, an amended test report will be generated and provided to the referring physician, when possible. A change in interpretation may be due to new evidence that indicates a variant is more or less likely to be deleterious than indicated by evidence existing at the time of initial reporting.