

# Drug Induced Long QT Test Technical Specifications

Cogenics Drug-Induced Long QT test can identify genetic variants in five ion channel genes that increase susceptibility to arrhythmias associated with Long QT Syndrome (LQTS). This test can also identify genetic variants in SCN5A, a cardiac channel gene associated with Brugada Syndrome, progressive cardiac conduction disease, dilated cardiomyopathy and congenital sick sinus syndrome; and in KCNQ1 and KCNH2, the two cardiac channel genes thus far associated with Short QT Syndrome (SQTS).

## DESCRIPTION OF GENETIC ASSAYS

The test can be ordered in 2 configurations:

**1. Comprehensive Cardiac Ion Channel Analysis.** Provides analysis for variants in five major cardiac ion channel genes. This analysis includes sequence determination and variant detection in open reading frame (ORF) and intronic sequences containing splice junction sites. The following genes are analyzed:

GENE	CURRENT	BASE-PAIRS ANALYZED	PCR AMPLICONS
KCNQ1/ LQT1	$I_{Ks}$	2331	17
KCNH2/ LQT2	$I_{Kr}$	3750	18
SCN5A/ LQT3/BS1	$I_{Na}$	6571	34
KCNE1/LQT5	$I_{Ks}$	390	2
KCNE2/LQT6	$I_{Kr}$	372	2

**2. Sodium Channel Analysis.** Provides analysis for variants only for the SCN5A gene. This analysis includes sequence determination and variant detection in ORF and intronic sequences containing splice junction sites.

## DESCRIPTION OF METHODS

**1. Sample acquisition:** 20 ml blood sample in two (2) 10 ml EDTA tubes should be shipped overnight at room temperature to Cogenics. Genomic DNA is isolated from fresh whole blood from each patient sample. Bar codes on each tube provide sample tracking

**2. DNA Sequence Analysis:** DNA amplification of the patient DNA 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 ORF, splice junction sites and flanking regions for each gene. Each amplicon is produced in two independent PCR reactions and directed sequencing is performed in both forward and reverse directions using dye-terminator chemistries. Automated electrophoretic separation of sequencing reactions is performed. At least one forward and one reverse read are required for each amplicon.

**3. Variant Detection:** Sequence traces are analyzed for heterozygous or homozygous variants with respect to a reference sequence. This reference sequence is based on the publicly available sequence and has been confirmed in a sampling of several hundred individuals of diverse ancestry. For each amplicon, the analysis uses two reference traces (forward and reverse) that match the reference sequence exactly. The reference traces

were generated using the same protocol as the patients' traces. Patient sequence traces are visually compared with reference traces to validate variant calls. In order to be analyzed, each trace must meet a quality metric of less than 1 error per 1,000 bases at every homozygous position. Two trained technicians independently score all traces for variants and a trained supervisor reconciles discrepancies.

**4. Report Generation:** Class I, II, and III variants, as defined below, are reported.

## PERFORMANCE CHARACTERISTICS

### 1. Analytical Specificity (Comprehensive or Sodium Channel):

The chance of a falsely detected genetic variant is minimized by requiring that each variant be seen in sequence traces for both forward and reverse directions and that two trained technicians independently examine each trace. Chances of false positives are minimized by the use of a validated sample tracking system that uses robotics and barcodes. 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

### 2. Analytical Sensitivity (Comprehensive or Sodium Channel):

Failure to detect a variant in an analyzed amplicon could be due to that 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 five genes account for 50-75% of cases of familial LQTS and 15-30% of Brugada Syndrome (Splawski et al. Circulation 102:1178-1185. 2000; Wilde et al. Circulation 107:2514-2519. 2002; Tester et al. Heart Rhythm, 2:507-517. 2005).

**4. Clinical Specificity:** It is estimated that 5% of apparently unaffected individuals test positive for a possible or probable deleterious mutation in one of these five genes, while approximately 3% of apparently unaffected individuals test positive for a possible or probable deleterious mutation in SCN5A alone (Ackerman et al. Mayo Clin Proc 78:1479-1487. 2003; Ackerman et al. Heart Rhythm 1:600-6107. 2004).

**5. Limitations:** There may be amplicons for which it is not possible to generate traces in both directions. This assay 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.

## VARIANT CLASSIFICATION

DNA variants in patient samples are identified and classified by comparison with reference sequences and the PGxHealth Cardiac Ion Channel Variant Database. This database is produced through review of published literature and GenBank references. The Cardiac Ion Channel Variant Database is continually updated and curated by expert reviewers. This database also contains an extensive collection of common polymorphisms and rare variants in these five genes that are not expected to confer susceptibility to LQTS or Brugada Syndrome; these variants were found in comprehensive scanning of the five genes in a over 1300 individuals of diverse race and ethnicity, the "Reference Panel." The individuals in the Reference Panel were not known to have LQTS, Brugada Syndrome, or related syndromes (Ackerman et al. Mayo Clin Proc 78:1479-1487. 2003; Ackerman et al. Heart Rhythm 1:600-6107. 2004).

## INTERPRETIVE CRITERIA

Each variant that is detected is categorized into one of 4 classes based on the type and location of the variant as described below. Note that these classifications should not be confused with the designations LQT1, LQT2, LQT3, LQT5 and LQT6, which refer to the affected gene in LQTS (see Description of Genetic Assays).

### CLASS I: Deleterious and Probable Deleterious Mutations

1. Functionally characterized as abnormal
2. Non-synonymous single nucleotide polymorphism (SNP) variant not seen in the Reference Panel.  
(A non-synonymous variant causes a change in the encoded amino acid.)
  - a. Nonsense variant
  - b. Transmembrane-spanning domain or pore
3. Insertion/Deletion
  - a. Frameshift variant with premature truncation
  - b. In-frame deletion/insertion localizing to transmembrane spanning domain or pore

### CLASS II: Possible Deleterious Mutations

1. Non-synonymous, missense SNP variant not seen in reference panel localizing to either N-terminal domain, C-terminal domain, or Na Channel linker (DI-DII, DII-DIII, DIII-DIV)
2. Non-synonymous, missense variant seen in the Reference Panel with allelic frequency < 0.5% and seen in at least one published case
3. In-frame deletion/insertion localizing to either N-terminal domain, C-terminal domain, or Na Channel linker (DI-DII, DII-DIII, DIII-DIV)
4. Splice site variant:
  - a. Intronic splice site variant
  - b. Variant in the last two nucleotides of an exon

### CLASS III: Variants Not Generally Expected to be Deleterious

1. Non-synonymous variant seen in the reference panel with either
  - a. Common frequency (> 0.5%) or
  - b. < 0.5% frequency but not published in LQTS literature

### CLASS IV: Non-Protein-Altering Variants

All non-coding and synonymous variants (no changes in encoded amino acid) except those in positions involved in 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.

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This test is performed by Cogenics, Inc., 5 Science Park, New Haven, CT 06511.

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