PGx HEALTH™
A DIVISION OF CLINICAL DATA

PGxPredict:™CLOZAPINE
TECHNICAL SPECIFICATIONS

The results of this test should be interpreted and utilized after review of the following specifications:

PRODUCT INDICATION
The PGxPredict:CLOZAPINE test is intended for use as an adjunct to clinical information in ascertaining the risk of clozapine-induced agranulocytosis (CIA) and the risk-benefit ratio of clozapine treatment. This test assays a single nucleotide polymorphism (SNP) in *HLA-DQB1* in whole blood. The use of this test does not eliminate the need for monitoring of white blood cell counts as recommended in clozapine prescribing information.

DESCRIPTION OF GENETIC ASSAYS
One SNP in intron 4 of the gene *HLA-DQB1* associated with CIA will be analyzed: 6672G>C.

DESCRIPTION OF METHODS
1. Sample Acquisition: Whole blood is collected via finger stick on to two FTA® Cards (Whatman®, Middlesex, UK) from which genomic DNA is isolated. Bar code identifiers are used to identify and track samples through a laboratory information management system.
2. DNA Sequence Analysis: The DNA is amplified by polymerase chain reaction (PCR), and is used to generate a template for direct sequencing. The amplicon is produced in two independent PCR reactions and direct sequencing is performed in both forward and reverse directions using dye-terminator chemistries. Automated electrophoretic separation of sequencing reactions is performed. At least two reads are required for the region.
3. Variant Detection: Sequence traces are analyzed for heterozygous or homozygous variants with respect to a publicly available reference sequence. The patient and reference traces are generated using the same protocol. Patient sequence traces are compared with reference traces to validate variant results. Two trained technicians independently score all traces for variants, and a trained supervisor reconciles discrepancies.
4. Report Generation: The CIA susceptibility-associated variant is reported, and patients are classified as "Lower Risk" or "Higher Risk" for CIA according to the table below. The final report is reviewed and signed by a CLIA-licensed Laboratory Director.

PERFORMANCE CHARACTERISTICS
Analytical Accuracy: The test is 99.8% accurate in identifying the genotype of the 6672G>C SNP in *HLA-DQB1*. The chance of an inaccurate genotype is minimized by requiring that the 6672G>C genotype be determined in sequence traces for both forward and reverse directions, and that two trained technicians independently examine each trace. Chances of inaccurate results are further minimized by the use of a validated sample tracking system that uses robotics and bar code identifiers.

This test was developed and its performance characteristics determined by PGxHealth, LLC. FDA approval is not currently required for clinical use of this test. Validation was done as required by the Clinical Laboratory Improvement Amendments of 1988 (CLIA) (CLIA License Number: 07D0995237; State License Number: CL-0633).

TEST INTERPRETATION
Based on data from 206 patients in two independent cohorts, the HLA-DQB1 6672G>C genotype is utilized to classify patients as Lower or Higher Risk of CIA, as described in the table below. This test has a sensitivity is 21.5% and specificity is 98.4%.

The genotypes will be interpreted according to the following table:

6672G>C Genotype	Risk	Interpretation Paragraph
GG	Lower	Testing indicates the absence of a high-risk genotype in the gene *HLA-DQB1*. This result predicts a slightly lower than average risk of agranulocytosis associated with clozapine use. Patients classified as Lower Risk have an estimated risk of CIA of 0.32% (1 in 313 patients), a 20% risk reduction from the overall patient population risk of 0.4% observed with blood monitoring.[i]
GC or CC	Higher	Testing indicates the presence of a high-risk genotype in the gene *HLA-DQB1*. This result predicts a higher than average risk of agranulocytosis associated with clozapine use. Patients classified as Higher Risk have an estimated risk of CIA of 5.1%, an 1175% risk elevation from the overall patient population risk of 0.4% observed with blood monitoring.[i]

[i] Clozaril® (Novartis) prescribing information (last revision March 2008)

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