AutoGenomics, Inc.

AvertD®

Package Insert
Instructions For Use

For *In Vitro* Diagnostic Use
For Prescription Use Only

- **Opioid-sparing techniques should be used when prescribing oral opioids for all patients, regardless of test result.**
- **Not for use in patients receiving treatment for chronic pain.**
- **An Elevated Genetic Risk test result does not mean that a patient will develop OUD or does not already have OUD. Results from this test are not intended for the diagnosis of OUD.**
- **A Non-Elevated Genetic Risk test result does not mean that a patient will not develop OUD.**
- **Results of the test should not be used alone to make any decisions regarding treatment. Results may be used as part of a complete clinical evaluation and risk assessment to determine appropriate pain management strategies.**
- **This test is intended for voluntary use. It is not intended for use as part of a mandated testing program.**

1 INTENDED USE

AvertD® is a prescription, qualitative genotyping test used to detect and identify 15 genetic polymorphisms in genomic DNA isolated from buccal or blood samples collected from individuals 18 years of age and older. The test may be used as part of a clinical evaluation and risk assessment to identify patients who may be at elevated risk for developing Opioid Use Disorder (OUD). The test is indicated for use only in patients prior to receiving a first prescription of oral opioids for 4-30 days for acute pain, such as in patients scheduled to undergo a planned surgical procedure and who consent to having the test performed.

2 BACKGROUND INFORMATION

Fifteen percent of US adults filled one or more opioid prescriptions in 2018 (CDC 2019), typically for pain relief. Although opioids may be of benefit in the treatment of acute and chronic pain when used as prescribed, even when used as prescribed, use of opioids can lead to dependence, misuse, and addiction in some people. A combination of several poorly understood factors (including genetic and non-genetic factors) may contribute to opioid misuse and abuse.

Opioid misuse and abuse have become a public health crisis in the US, associated with significant mortality, morbidity, and expense:

a. Drug overdose is the leading cause of death for Americans under 50 and opioids account for most overdose deaths (Drug Policy Alliance 2020).
b. Every day, more than 130 Americans die from opioid addiction (CDC. America's Drug Overdose Epidemic: Data to Action 2020).
c. Opioid overdose causes approximately 185,000 Emergency Room Department visits annually for patients aged 15 and older (CDC 2017).
d. Opioid misuse and abuse disproportionately impact young-adult Americans, with peoples aged 26-34 years old having the highest percentage of self-reported prescription pain reliever misuse and abuse (CDC 2019).
e. The total US economic burden of Opioid Use Disorder (OUD - problematic use of opioids leading to impairment or distress) was estimated to be a staggering $179 billion in 2018 (Davenport 2019).

Effectively addressing this public health crisis will require many different interventions, including more selective use of opioids for acute pain relief. Research has indicated that there may be genetic factors that contribute to a person's risk for developing OUD; however, genetic associations of individual genes identified so far explain only a small portion of OUD risk. AvertD® is a test that identifies genetic variants and uses the presence or absence of these variants to assess genetic risk of developing OUD. Information from AvertD® may be used as part of a clinical evaluation and risk assessment to provide patients and healthcare providers with information that can be used to aid in treatment decision-making prior to the first prescription of oral opioids for 4 to 30 days for acute pain, such as in patients scheduled to undergo a planned surgical procedure, and who consent to having the test performed.

3 TEST PRINCIPLE/ASSAY OVERVIEW

AvertD® utilizes hybridization capture array with automated detection of multiplex PCR products. AvertD® is designed to identify 15 genetic polymorphisms in genomic DNA isolated from human buccal or blood samples. AvertD® identifies and uses the presence or absence of these variants to assess genetic risk of developing OUD.
The following are the 15 genetic polymorphisms detected by AvertD®.

Allelic Variants	Gene Name	rs Number
5-HTR2A C>T	Serotonin 2A Receptor	rs7997012
COMT G>A	Catechol-O-Methyltransferase	rs4680
DRD1 A>G	Dopamine D1 Receptor	rs4532
DRD2 G>A	Dopamine D2 Receptor	rs1800497
DRD4 T>C	Dopamine D4 Receptor	rs3758653
DAT1 A>G	Dopamine Transporter	rs6347
DBH C>T	Dopamine Beta Hydroxylase	rs1611115
MTHFR C>T	Methylene Tetrahydrofolate Reductase	rs1801133
OPRK1 G>T	Kappa Opioid Receptor	rs1051660
GABA C>A	Gamma-Aminobutyric Acid (GABA)	rs211014
OPRM1 A>G	Mu Opioid Receptor	rs1799971
MUOR G>A	Mu Opioid Receptor	rs9479757
GAL T>C	Galanin	rs948854
DOR G>A	Delta Opioid Receptor	rs2236861
ABCB1 C>T	ATP Binding Cassette Transporter 1	rs1045642

AvertD® involves the following processes:

a) Buccal swab sample collection using the AvertD® Buccal Sample Collection Kit or blood sample collection using BD Vacutainer® K2EDTA Blood Collection Tubes
b) DNA extraction from the buccal or blood samples
c) Multiplex PCR amplification of DNA
d) SAP/EXO processing for combined amplified products
e) Fluorescent label incorporation using analyte specific primer extension (ASPE)
f) Hybridization of the ASPE primers to a microarray followed by washing
g) Scanning of the microarray
h) Signal detection and analysis

Steps (e) through (h) are automated on the INFINITI® PLUS.

The intensity of the signal indicates the presence or absence of the target analytes in the samples. This information is processed by an algorithm that is used to assess genetic risk of developing OUD following acute exposure to prescription oral opioids.

The AvertD® test report includes the score determined by the algorithm and the genetic risk for OUD as "Elevated Genetic Risk", "Non-Elevated Genetic Risk", or "N/A" when a score cannot be determined.

4 DEVICE DESCRIPTION

AvertD® utilizes proprietary film-based microarray technology for multiplex detection of the 15 genetic polymorphisms.

AvertD® is comprised of the following:

- BioFilmChip® Microarray
- Intellipac® Reagent Module
- Amplification Mix
- Assay Protocol and Header

The **BioFilmChip® Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have a versatile surface to enhance test performance. Each microarray is designed to be assay-specific.

The **Intellipac® Reagent Module** acts as a communication link with up to four reservoirs that house the test reagents. This module has an integrated memory chip that stores reagent information such as lot number, expiration date, and number of tests.

The **Amplification Mix** provides the reagent for the PCR amplification of the DNA sample.

The OUD GAP/Header contains the **Assay Protocol** (GAP), which specifies the assay steps, parameters, and conditions, and the assay **Header,** which specifies the algorithm, assay multipliers, and ratios/cut-offs. The OUD GAP/Header is loaded into the INFINITI® PLUS, which performs the assay.

The **INFINITI® PLUS** is an instrument for measuring and sorting multiple signals from clinical samples. The INFINITI® PLUS measures fluorescence signals of labeled DNA target hybridized to BioFilmChip® microarrays. It integrates the discrete processes of sample (PCR amplicon) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and the spots are read by the built-in confocal microscope. The INFINITI® PLUS results include the genotype calls and the patient's genetic risk for OUD (reported as "YES", "NO", or "N/A" when genetic risk cannot be determined).

Instructions for using the INFINITI® PLUS are provided in the INFINITI® PLUS Operator's Manual.

5 WARNINGS AND PRECAUTIONS

Handling Requirements

- For *in vitro* diagnostic use by qualified laboratory personnel.
- For use with human buccal swab samples collected using the AvertD® Buccal Sample Collection Kit or with blood samples collected with BD Vacutainer® K2EDTA Blood Collection Tubes.
- Buccal swab specimens should be stored at 18 - 24 °C the time indicated on the AvertD® Buccal Sample Collection Kit labeling.
- Whole blood samples should be stored at 18 - 24 °C and used withing 14 days of collection.
- All patient samples are potentially hazardous, and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV, or other infectious agents are absent.

Whole blood shipping conditions for AvertD samples are defined as follows.

Temperature requirements during transport:

- Samples should be transported at 15 - 30 °C.
- Samples should not be frozen.

Maximum shipping duration and time limits:

- Whole blood samples should be shipped overnight within 72 hours of collection when stored and transported at 15 - 30 °C.

Packaging requirements and specifications: Samples must be packaged and transported in accordance with applicable regulations for Biological Substance, Category B (UN3373), including:

- Leak-proof primary container (BD Vacutainer® tube)
- Absorbent material surrounding the primary container
- Secondary leak-proof container
- Rigid outer shipping container with appropriate labeling

Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).

- Upon receipt of samples, visually inspect sample condition. Specifically, look for signs indicating that sample integrity may have been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.

- Handle samples with extreme caution to prevent contamination, spillage, and sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Perform sample preparation, PCR reaction set up, and PCR product analysis according to approved guidelines such as the CLSI Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline, which will minimize the risk of cross contamination.
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow standard laboratory precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves, laboratory coats) and eye protection; do not eat, drink, or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, samples, and waste according to applicable country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available upon request from Customer Service.

Sample Preparation

- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- Perform the PCR properly, ensuring proper pipetting of reagents.
- Ensure proper sealing of the PCR tubes by pressing down on the lid.
- Visually inspect each PCR product for indication of evaporation (e.g., low volume or discoloration).
- The PCR product must be used immediately. It cannot be stored prior to loading onto the INFINITI® PLUS.

INFINITI® PLUS

- **Read the Operator's Manual before operating the instrument.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

6 STORAGE / STABILITY

BioFilmChip® Microarray:	12 months Refrigerated (2 to 8ºC)
Intellipac® Reagent:	12 months Refrigerated (2 to 8ºC) ***Note:*** Do not use after Intellipac® has been opened for three weeks
Amplification Mix:	12 months Frozen (-30 to -15ºC)

Note: Specific product expiration date is printed on the product label

7 MATERIALS PROVIDED (SUFFICIENT FOR 48 TESTS)

- Product Number 03-1540-01 AvertD® BioFilmChip® Microarray Magazine:
  4 magazines per package
  12 microarray chips per magazine
- Product Number 03-2540-01 AvertD® Intellipac® Reagent Module:
  2 modules per package
  24 tests per module

  Each Intellipac® module contains:

  1.1ml ASPE Master Mix:
  Extension Reaction Buffer
  Labeled-dCTP
  dNTPs
  Allele Specific Primers

  2. 6ml Hybridization Buffer:
  SSC
  EDTA
- Product Number 03-3540-01 AvertD® Amplification Mix:
  2 x 500µl vials of amplification mix

  Amplification Mix contains
  Multiplex Primer Mix
  dNTPs
  PCR Buffer
- Product Number 12-0330-00: Wash buffer

8 REAGENTS REQUIRED BUT NOT PROVIDED

- DNA Extraction Kits- AvertD® can detect the 15 genetic polymorphisms using genomic DNA, isolated from buccal or blood samples, with sufficient purity. DNA should have an absorbance ratio $A_{260}/A_{280} \geq 1.2$ and DNA concentration ≥ 1 ng/µl and up to 60 ng/µl. Genomic DNA samples with concentrations between 60 ng/µl and 240 ng/µl should be diluted with 1× TE buffer to within the recommended upper input range prior to AvertD testing. Any DNA extraction method that meets this specification may be used. AvertD® has been tested with several commercially available kits. Contact SOLVD Health for further information.
- Titanium Taq DNA Polymerase (Clontech Catalog # 639209)
- Shrimp Alkaline Phosphatase (SAP, Affymetrix, Catalog # 78390)
- Exonuclease I (Exo I, Affymetrix, Catalog # 70073X)
- Distilled water (DNAse and RNAse free)

9 EQUIPMENT

The following equipment is required but not provided with the assay reagents:

- INFINITI® PLUS (Catalog # 10-0020-01)
- INFINITI® Pipette Tips (Catalog # 11-0080-00)
- INFINITI® Waste Tray Liners (Catalog # 11-0020-00)
- INFINITI® Waste Tray Stir Bars (Catalog # 11-0060-00)
- FOR INFINITI® ANALYZER: INFINITI® Temp Cycler Plates (Catalog # 11-0050-00)

- FOR INFINITI® PLUS ANALYZER: INFINITI® 48 Well Plate (Catalog # 11-0100-00) and 48 Well Lid (Catalog # 11-0110-00)
- DNA Extraction Kit (Document Manufacturer and Catalog Number)
- Thermocyclers: Eppendorf Mastercycler® Pro with aluminum block; Eppendorf Mastercycler® X50s with silver block or Mastercycler® X50i with silver block
- 96-well PCR Plate (Eppendort twin.tec PCR 96, PN951020303 or 0030133404)
- PCR Adhesive Sealing Film (for Eppendorf plates, PN 30127781, for VWR plates VWR Catalog # 60941-076)
- Pipettors
- Mini Centrifuge
- Microfuge Tube Racks
- Vortex
- 1.5 ml Microcentrifuge Tubes

10 REQUIREMENTS

AvertD® can be used with buccal samples collected using the AvertD® Buccal Sample Collection Kit. Buccal samples should be extracted following the manufacturer's instructions for the DNA extraction kit. Samples that have been collected within 12 months, and which have an absorbance ratio $A_{260}/A_{280} \geq 1.2$ and a DNA concentration ≥ 1ng/µl and up to 60 ng/µl may be used for AvertD®.

AvertD® can also be used with whole blood samples collected with BD Vacutainer® K2EDTA Blood Collection Tubes. Whole blood samples should be processed following the manufacturer's instructions for the DNA extraction kit. Whole blood samples that have been collected within 14 days and have an absorbance ratio $A_{260}/A_{280} \geq 1.2$ and a DNA concentration ≥ 1 ng/µl and ≤ 60 ng/µl may be used for AvertD®.

Genomic DNA samples with concentrations between 60 ng/µl and 240 ng/µl should be diluted with 1× TE buffer to within the recommended upper input range prior to AvertD testing, following the scheme outlined below, without impacting assay performance.

Initial DNA Conc. (ng/µL)	Ratio (DNA:Total)	Example Mix
≥1 ng/µL, ≤ 60 ng/µL	no dilution	N/A
>60 ng/µL, ≤ 120 ng/µL	1: 2	10 µL DNA + 10 µL 1X TE buffer
>120 ng/µL, ≤ 180 ng/µL	1: 3	10 µL DNA + 20 µL 1X TE buffer
>180 ng/µL, ≤ 240 ng/µL	1: 4	10 µL DNA + 30 µL 1X TE buffer

11 QUALITY CONTROL

A known positive control (heterozygous and/or homozygous samples) and a negative control (i.e., wild type sample) should be included in each test run, along with a non-template control equivalent (NTCE) which is expected to give No-Calls. This NTCE control demonstrates that reagents are free of contaminants which may impact testing results. Well-characterized DNA samples are suitable positive controls for the detected genotypes. Please contact SOLVD Health for recommendations on use sources of well-characterized DNA.

Note: The thermocycler used should be regularly maintained and calibrated with an external temperature standard, according to the laboratory's regulatory and QC requirements.

12 DNA EXTRACTION

Follow the instructions provided with the DNA extraction kit used.

13 AMPLIFICATION PROCEDURE WITH EPPENDORF MASTERCYCLER PRO



Mastercycler Pro

Note: (a) Keep Titanium Taq DNA polymerase on ice.

(b) Completely thaw reagents at room temperature.

(c) Vortex the amplification mix tube for 2 to 5 seconds. Then centrifuge briefly to bring the contents to the bottom of the tube.

(d) To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach in de-ionized or distilled water. Filter tips and gloves must be used when handling specimens and controls.

(e) Prior to amplification, ensure the PCR tubes are adequately sealed with the flat caps to prevent evaporation during thermocycling.

13.1 Prepare the PCR master mix

Amplification mix	17.8 µl
Titanium Taq polymerase	0.2 µl
Total volume of PCR Master Mix	18.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

13.2 Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the 96-well PCR Plate .

13.3 Add 2 µl of sample DNA to each well.

PCR master mix	18.0 µl
Sample DNA	2.0 µl
Volume of amplification reaction	20.0 µl

13.4 Seal the 96-well plate with PCR Adhesive Sealing Film and place it in a thermocycler.

Immediately commence the amplification reaction using the following program.

Step No.	Temperature °C	Time	No. of Cycles
1	98	5 min	1
2	94 69-60(-1.0/cycle)	15 sec 15 sec	10x
3	94 59	15 sec 15 sec	30x
4	94	15 sec	1
5	4	HOLD	1

Note: Step 1 is set at 100% ramp rate. After each cycle in step 2 the temperature is decreased by 1.0°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour (± 3 min). For other thermocycler models, adjust the ramp rate to obtain equivalent cycling time.

14 AMPLIFICATION PROCEDURE WITH EPPENDORF MASTERCYCLER X50s



Mastercycler X50s

Note: (a) Keep Titanium Taq DNA polymerase on ice.

(b) Completely thaw reagents at room temperature.

(c) Vortex the amplification mix tube for 2 to 5 seconds. Then centrifuge briefly to bring the contents to the bottom of the tube.

(d) To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach in de-ionized or distilled water. Filter tips and gloves must be used when handling specimens and controls.

(e) Prior to amplification, ensure the PCR tubes are adequately sealed with the flat caps to prevent evaporation during thermocycling.

14.1 Prepare the PCR master mix.

Amplification mix	17.8 µl
Titanium Taq polymerase	0.2 µl
Total volume of PCR Master Mix	18.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

14.2 Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the 96-well

plate.

14.3 Add 2 µl of sample DNA to each well.

PCR master mix	18.0 µl
Sample DNA	2.0 µl
Volume of amplification reaction	20.0 µl

14.4 Place the 96-well plate, sealed with PCR Adhesive Sealing Film, in a thermocycler and immediately commence the amplification reaction using the following program.

Step No.	Temperature °C	Time	No. of Cycles
1	98	5 min	1
2	94 69-60(-1.0/cycle)	15 sec 15 sec	10x
3	94 59	15 sec 15 sec	30x
4	94	15 sec	1
5	4	HOLD	1

Note: Run time, 1 hour; temperature mode, standard; block settings, silver 96. After each cycle in step 2 the temperature decreases by 1.0 ºC. Estimated ramp rate is 1.37 ºC/s.

15 PCR CLEAN UP

Post-PCR cleanup is a critical step to ensure the remaining substrates would not carry through and interfere with the signal amplification.

Note: Viscosity of the enzyme mixture will require slower pipetting.

15.1 Prepare the enzyme mixture as a master mix. For example, if there are 96 PCR reactions, create a master mix, enough for 100 reactions. Any leftover enzyme mix can be stored at -20°C for up to 6 months.

SAP (1U/µl)	1.50 µl
Exonuclease I (10U/µl)	0.375 µl
Titanium Taq Polymerase (50x)	0.125 µl
Total	2.0 µl

15.2 Dispense 2 µl of the enzyme mixture per reaction.

16 ASSAY PROCEDURE USING EPPENDORD MASTERCYCLER X50i



MASTERCYCLER X50i

The Mastercycler X50i operates exclusively as an extension unit and must be externally controlled, either by connecting it directly to a Mastercycler® X50s unit (which can centrally manage up to nine X50i units via its 7-inch touchscreen) or through a PC running Eppendorf CycleManager X50 software.

Despite the lack of an independent user interface, the Mastercycler® X50i features identical internal hardware and thermal performance specifications to the Mastercycler® X50s. Both units utilize a high-thermal-conductivity 96-well silver block capable of rapid heating ramp rates up to 10.0 °C/s and cooling rates up to 5.0 °C/s. This shared architecture—including advanced 2D-Gradient temperature optimization and an automated flexlid evaporation barrier—ensures absolute protocol reproducibility, identical PCR kinetics, and seamless assay transfer between the Mastercycler X50i and Mastercycler X50s units. To perform the AvertD assay with the Mastercycler X50i, follow the procedure as described for the Mastercycler X50s.

17 SAMPLE LOADING - INFINITI® PLUS

Transfer samples from 96 well PCR plate to INFINITI® 48 Well Plate 48WP with the 48WP lid (Catalog # 11-0110-00, reusable) in the appropriate orientation (with well A1 in the back left corner), assay specific Magazines, Intellipac®, static free pipette tips, and Buffer into the INFINITI® PLUS.

For operation of the INFINITI® PLUS, refer to the INFINITI® PLUS Operator's Manual (EM-34041).

18 INTERPRETATION OF RESULTS

18.1 Genotype Calls

Allelic Variants	rs Number	Genotypes		
		Wild	Mutant	Het
5-HTR2A C>T	rs7997012	CC	TT	CT
COMT G>A	Rs4680	GG	AA	GA
DRD1 A>G	rs4532	AA	GG	AG
DRD2 G>A	Rs1800497	GG	AA	GA
DRD4 T>C	rs3758653	TT	CC	TC
DAT1 A>G	rs6347	AA	GG	AG
DBH C>T	rs1611115	CC	TT	CT
MTHFR C>T	rs1801133	CC	TT	CT
OPRK1 G>T	rs1051660	GG	TT	GT
GABA C>A	Rs211014	CC	AA	CA
OPRM1 A>G	rs1799971	AA	GG	AG
MUOR G>A	rs9479757	GG	AA	GA
GAL T>C	rs948854	TT	CC	TC
DOR G>A	Rs2236861	GG	AA	GA
ABCB1 C>T	rs1045642	CC	TT	CT

18.2 AvertD® will determine and report the genetic risk score generated by the algorithm for developing OUD. The risk of OUD will be printed on the Results Report as "Elevated Genetic

Risk" if the patient has an elevated risk of OUD and "Non-Elevated Genetic Risk" if patient does not have an elevated risk of OUD.

18.3 Very rarely there could be an "IND" reported for a SNP, indicating an indeterminate SNP. This may be caused due to a novel genotype or SNP (not included in the panel) interfering with the genotype detection. When any of the 15 SNPs used in the algorithm is reported as "IND", the risk score will not be generated and the genetic risk for developing OUD will be reported as "N/A". The test should be repeated.

18.4 A "no call" will be reported if the signal is not qualified, i.e., relative fluorescent unit (RFU) used in variant detection is low. When any of the SNPs is reported as "no call", the risk score will not be generated and the genetic risk for developing OUD will be reported as "N/A". The test should be repeated.

18.5 DNA controls should make expected genotype calls for each allelic variant. The NTCE control should give "no calls" and the genetic risk for OUD will be reported as "N/A". If controls do not perform as expected, the run is considered invalid and should be repeated.

18.6 When the assay is not completed, and no genotype call is made, the assay will need to be repeated. The report displays a message which indicates the reason why no genotype call was made. When an error occurs (e.g., "low DNA"), an Error Log is generated which identifies the problem. Please refer to the Trouble Shooting section of the INFINITI® PLUS Operator's Manual.

19 LIMITATIONS

AvertD® test results indicate if a patient may have an elevated genetic risk of developing OUD based on the presence or absence of 15 specific SNPs. The AvertD® test was evaluated in a clinical study which assessed the test's ability to correctly classify OUD-positive patients and OUD-negative patients. **There are many known factors associated with increased risk for developing OUD, including genetic and non-genetic factors. There may be additional factors that may not have been recognized. The interactions among these factors are not well understood. While AvertD® assesses the genetic risk for OUD, it does not assess non-genetic factors associated with OUD risk. Therefore, AvertD® is intended to be used as part of a clinical evaluation and risk assessment for OUD that considers other factors. The actual risk of developing OUD for an individual patient may be higher or lower than that observed in the clinical study due to other factors not included in the AvertD® report.**

- It is important to follow opioid prescribing guidelines for all patients, including those who receive a Non-Elevated Genetic Risk result.
- Any use of opioids involves risk. Taking opioids without appropriate medical oversight is particularly risky.
- The test is not intended to be used in patients being treated for chronic pain.
- This test should only be used for patients 18 years of age or older.
- There are many known factors associated with increased risk for OUD, including genetic and non-genetic factors, along with additional factors that may not have been recognized. The interactions among these factors are not well understood.
- An Elevated Genetic Risk result does not mean an individual has or will develop OUD. Similarly, a Non-Elevated Genetic Risk result does not mean an individual does not have or will not develop OUD.
- Non-genetic factors associated with OUD risk are not included in the AvertD® risk assessment. The actual risk of developing OUD may be higher or lower due to other factors not included in the AvertD® report.
- The genetic test results in this report are intended for use solely by a qualified healthcare provider. Providers should use these results only in conjunction with a complete clinical evaluation.
- An individual should not make medical decisions, change their health behaviors, or make changes to medications or dosages without consulting their healthcare provider.
- This test is only to be used prior to the first prescription of oral opioids for a course of 4-30 days for the treatment of acute pain. If you are considering prescribing oral opioid treatment outside the 4-30 day timeframe, this test should not be used. If the patient may require long-term pain management with oral opioids, this test should not be used. If the patient has previously taken prescription oral opioids, this test should not be used.
- The clinical study assessed the device performance in a subset of the general population and results of the clinical study may not apply to all sub-populations in the US.

20 PERFORMANCE CHARACTERISTICS

20.1 Algorithm Development and Training

The AvertD® algorithm was developed and trained using a 5-fold cross-validation with 20% holdout in the training dataset. In the training dataset, 1,381 subjects were included with 479 subjects with OUD and 902 without OUD. The 1,381 subjects were all from the United States. Of the 1,381 subjects, age was available for 972 subjects with a mean age of 38.7 years (std dev: 9.9). Sex was available for 986 subjects: 33.9% were female, 37.5% were male, and

information was unavailable for 28.6%. The training dataset over-represented African-Americans compared to the US general population. In the training dataset, the category "Other" includes Asian-Americans, multiracial, mixed racial, and biracial Americans; these were the categories used to collect the data. In the training dataset, approximately 19% of the subjects were Hispanic, consistent with the US general population.

Table 19-1a. OUD Negative and OUD Positive Subjects by Race in the Training Dataset

Race	OUD Negative N (%)	OUD Positive N (%)	Totals
African-American	271 (30.0%)	154 (32.2%)	425
Other	123 (13.6%)	11 (2.3%)	134
White	337 (37.4%)	245 (51.1%)	582
Not Specified	171 (19.0%)	69 (14.4%)	240
Total	**902**	**479**	**1,381**

Table 19-1b. OUD Negative and OUD Positive Subjects by Ethnicity in the Training Dataset

Ethnicity	OUD Negative N (%)	OUD Positive N (%)	Totals
Hispanic	171 (19.0%)	101 (21.1%)	272
Non-Hispanic	731 (81.0%)	378 (78.9%)	1109
Total	**902**	**479**	**1,381**

20.2 Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. DPE primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

20.3 Limits of Detection (analytical sensitivity)

The analytical sensitivity (Limit of Detection or LOD) of AvertD® was assessed by testing 8 samples at 8 serial dilutions at 60 ng/ul, 30 ng/ul, 15 ng/ul, 7.5 ng/ul, 3 ng/ul, 1 ng/ul, 0.3 ng/ul, and 0.1 ng/ul of DNA. The genotypes of the samples tested were confirmed by bidirectional sequencing. A total of 1,280 tests were included in the study.

The limit of detection was defined as the lowest level of genomic DNA (ng DNA input per test) that would give a ≥ 95% correct call rate. The lower limit of detection was using DNA at a concentration of 1 ng/ul. At this lower limit, the percent correct call rate was 100.0%.

19.4 Assay Accuracy – Percent Agreement vs. Bidirectional Sequencing

AvertD® was compared to Sanger bidirectional sequencing to evaluate its accuracy in determining the genotype of the target analytes. Three laboratory sites participated in the study. Each site tested a different set of de-identified patient samples with AvertD®. Different DNA extraction methods were utilized by each site.

The results of the comparison study are shown below. AvertD® has an accuracy in determining the genotype of the target analytes of >99.95%.

Table 19-4: Agreement between AvertD® and Bidirectional Sequencing

Allelic Variants	Genotype	Accuracy of AvertD®	
		Number of Alleles with Concordance	Percentage of Alleles with Concordance
5-HTR2A (rs7997012) C>T	Wild Type	138/138	100.00%
	Heterozygous Mutant	236/236	100.00%
	Homozygous Mutant	60/60	100.00%
COMT (rs4680) G>A	Wild Type	119/119	100.00%
	Heterozygous Mutant	208/208	100.00%
	Homozygous Mutant	107/107	100.00%
DRD1 (rs4532) A>G	Wild Type	176/176	100.00%
	Heterozygous Mutant	196/196	100.00%
	Homozygous Mutant	62/62	100.00%
DRD2 (rs1800497) G>A	Wild Type	268/269	99.63%
	Heterozygous Mutant	151/152	99.34%
	Homozygous Mutant	13/13	100.00%
DRD4 (rs3758653) T>C	Wild Type	274/274	100.00%
	Heterozygous Mutant	146/146	100.00%
	Homozygous Mutant	14/14	100.00%
DAT1 (rs6347) A>G	Wild Type	235/236	99.58%
	Heterozygous Mutant	167/168	99.40%
	Homozygous Mutant	30/30	100.00%
DBH (rs1611115) C>T	Wild Type	276/276	100.00%
	Heterozygous Mutant	138/138	100.00%
	Homozygous Mutant	20/20	100.00%
MTHFR (rs1801133) C>T	Wild Type	197/197	100.00%
	Heterozygous Mutant	193/193	100.00%
	Homozygous Mutant	44/44	100.00%
OPRK1 (rs1051660) G>T	Wild Type	340/340	100.00%
	Heterozygous Mutant	88/88	100.00%
	Homozygous Mutant	6/6	100.00%
GABA (rs211014) C>A	Wild Type	260/260	100.00%
	Heterozygous Mutant	154/154	100.00%
	Homozygous Mutant	20/20	100.00%
OPRM1 (rs1799971) A>G	Wild Type	320/320	100.00%
	Heterozygous Mutant	100/100	100.00%
	Homozygous Mutant	14/14	100.00%

Allelic Variants	Genotype	Accuracy of AvertD®	
		Number of Alleles with Concordance	Percentage of Alleles with Concordance
MUOR (rs9479757) G>A	Wild Type	370/370	100.00%
	Heterozygous Mutant	60/60	100.00%
	Homozygous Mutant	4/4	100.00%
GAL (rs948854) T>C	Wild Type	229/229	100.00%
	Heterozygous Mutant	167/167	100.00%
	Homozygous Mutant	38/38	100.00%
DOR (rs2236861) G>A	Wild Type	250/250	100.00%
	Heterozygous Mutant	159/159	100.00%
	Homozygous Mutant	25/25	100.00%
ABCB1 (rs1045642) C>T	Wild Type	91/92	98.91%
	Heterozygous Mutant	218/219	99.54%
	Homozygous Mutant	123/123	100.00%

19.5 A Matrix Comparison of Paired Buccal and Blood Samples

A study was conducted to evaluate blood as an alternative sample type by bridging its performance against the approved buccal sample reference in a matrix comparison study using paired buccal swab and blood sample from 176 individuals**.** As shown in **Table 19.5**, 100% concordance of AvertD® results was observed for the paired buccal and blood samples of these 176 individuals.

Table 19.5 100% Concordance of AvertD® results from paired buccal and blood samples

AvertD genotype results of paired blood and buccal specimens											
	Buccal Specimens			Blood Specimens			Concordance: paired of buccal and blood specimens				
Analyte	Sample tested	Samples w/ invalid tests	Samples w/ Valid Tests	Sample tested	Samples w/ invalid tests	Samples w/ Valid Tests	Paired blood/buccal with valid results	Paired blood/buccal with invalid results	Paired specimens with cordant calls	% Concordance of paired samples	95% Confidence Intervals
5-HTR2A	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
COMT	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DRD1	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DRD2	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DRD4	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DAT1	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DBH	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
MTHFR	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
OPRK1	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
GABA	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
OPRM1	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
MUOR	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
GAL	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
DOR	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%
ABCB1	178	2	176	178	0	178	176	0	176	100	97.9%- 100.00%

19.6 Assay Inter-Laboratory Reproducibility

A three-site study was conducted to demonstrate the reproducibility of AvertD®. The study involved three reagent lots of AvertD®, two operators per site, three instruments (one per site), and three extraction methods.

The sites ran 12 identical samples and were masked to sample identity. At each site, each sample was run in duplicate per day/operator for 5 non-consecutive days. The 12 samples underwent bidirectional sequencing to confirm the genotype. From each of these 12 samples, three aliquots were sampled and sent to the sites to test using AvertD®.

Site 2 and Site 3 performed 240 tests each (12 samples x 5 days x 2 operators x 2 lots = 240 tests). Site 1 performed 245 tests. Each of the 15 analytes was tested 725 times. No repeats were allowed for the reproducibility study. The overall correct call rate was 100.0% with a 95% one-sided confidence limit of 100.0%. **Table 19-6** provides a summary of the reproducibility study results.

Table 19-6: AvertD® Reproducibility by Genotype

Analytes	Samples Tested	Samples with Invalid Tests	Samples with Valid Results	Valid Samples with Discordant Calls	Valid Samples with Concordant Calls	Percent Concordant Calls
5-HTR2A	725	30	695	0	695	100.00%
COMT	725	30	695	0	695	100.00%
DRD1	725	30	695	0	695	100.00%
DRD2	725	30	695	0	695	100.00%
DRD4	725	30	695	0	695	100.00%
DAT1	725	30	695	0	695	100.00%
DBH	725	30	695	0	695	100.00%
MTHFR	725	30	695	0	695	100.00%
OPRK1	725	30	695	0	695	100.00%
GABA	725	30	695	0	695	100.00%
OPRM1	725	30	695	0	695	100.00%
MUOR	725	30	695	0	695	100.00%
GAL	725	30	695	0	695	100.00%
DOR	725	30	695	0	695	100.00%
ABCB1	725	30	695	0	695	100.00%
Total	**10,875**	**450**	**10,425**	**0**	**10,425**	**100.00%**

To confirm reproducibility across all genotypes at DNA concentration at or near the limit of detection, an additional reproducibility study was conducted with 15 samples. The 15 samples included all gene variants. The samples were tested for 5 non-consecutive days on 3 instruments. The study results are consistent with the previous reproducibility study with 100% correct call rate.

19.7 Interfering Substances – Endogenous and Exogenous Substances

Buccal Swabs Samples

A study was conducted to evaluate the effect of potential endogenous and exogenous interfering substances on the performance of AvertD®. Buccal swab samples collected according to the instructions for use, from individuals who have been directly exposed to the potential exogenous interferents, were tested using AvertD®. The potential endogenous substance (whole blood) was added directly to the tube containing the stabilizing solution immediately prior to insertion of the buccal swab sample.

No interference with AvertD® was observed for any of the tested substances, which included: antiseptic mouthwash, toothpaste, baking soda, cough syrup, cranberry juice, table salt, sugar, meat, chewing gum, hard candy, cigarettes, coffee, and whole blood.

Whole Blood Samples

A study was conducted to evaluate the effect of potential endogenous and exogenous interfering substances on the performance of AvertD® whole blood samples collected with BD Vacutainer® K2EDTA Blood Collection Tubes. The potential endogenous substances were added directly to the whole blood sample immediately prior to DNA extraction.

No interference with AvertD® was observed for any of the tested substances, which included: hemoglobin (human), bilirubin, triglycerides, uric acid, acetaminophen, ethanol and bleach.

19.8 Sample Carry-Over

No sample carry-over was detected when 120ng of a positive sample followed by 6ng of a second positive sample and 120ng of a third positive sample was followed by a "No Template Control." This series of sample testing was repeated 12 times. A total of 48 tests using AvertD® were run. No sample carry-over was reported.

19.9 Clinical Performance

A multi-center US clinical study was conducted with the intention of evaluating the ability of the AvertD® assay to discriminate patients at elevated genetic risk for developing OUD from patients with non-elevated genetic risk using buccal swab samples collected from consenting patients. This clinical study was a blinded, multi-center study of patients with a history of exposure to prescription oral opioids. For each patient, a clinical assessment was performed to diagnose OUD at the time of enrollment which was at least 12 months (on average 10 years) following a self-reported index exposure to prescription oral opioid use. Each patient's confirmed OUD status was compared to the AvertD® test result.

812 patients were enrolled into clinical study from 10 clinical sites, and 385 patients were selected by a statistician for inclusion in study analyses. All patients were evaluated for OUD using a DSM-5 based clinical evaluation. One central laboratory tested all study samples, which contained study subject ID as the only identifier.

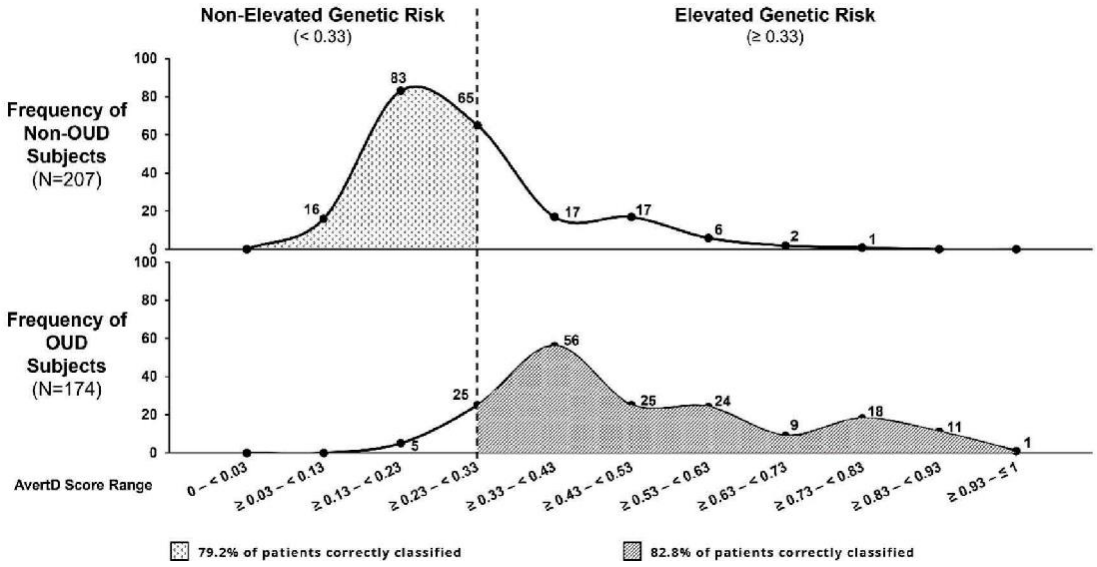
Of the 385 patients, AvertD® results were available for 381 (99%). Test results were not available for 4 patients due to inadequate DNA extraction from the buccal sample.

Risk Score and Cut-off: The AvertD® algorithm produces a continuous test result value from 0.000–1.000. A score ≥ 0.33 is the cut-off value for Elevated Genetic Risk for OUD

whereas a score < 0.33 is Non-Elevated Genetic Risk.

Figure 1 shows the distribution of AvertD® test scores for patients in clinical study with and without OUD; the cut-off value is represented by the dotted line. The further a patient's AvertD® score is from the cut-off value (smaller or greater), the more likely the patient was properly classified.

Figure 1. Distribution of AvertD® Test Scores for OUD Positive and OUD Negative Patients



Sensitivity and Specificity: Using the cut-off value, AvertD® had a sensitivity of 82.8% to correctly classify patients who were diagnosed with OUD with Elevated Genetic Risk of OUD and specificity of 79.2% to classify patients who were not diagnosed with OUD with Non-Elevated Genetic Risk (see **Table 19-9a**).

Table 19-9a: Sensitivity and Specificity of AvertD®

AvertD® Result*	OUD Status		Performance	Point Estimate	Exact 95% CI	
	OUD Positive	OUD Negative			Lower Bound	Upper Bound
Positive	144 (82.76%)	43 (20.77%)	Sensitivity	82.76%	76.31%	88.05%
Negative	30 (17.24%)	164 (79.23%)	Specificity	79.23%	73.06%	84.54%

The false positive rate observed in the study was 20.77% (43/207 incorrectly identified as Elevated Genetic Risk) and the false negative rate observed in the study was 17.24% (30/174 incorrectly identified as Non-Elevated Genetic Risk).

A sensitivity analysis was performed for the 4 patients without a test result. In the sensitivity analysis, 1 of the 4 patients was OUD positive and imputed as a negative test result (assuming this is a false negative) and 3 patients were non-OUD and imputed as false positives. Under these worst-case assumptions that all 4 missing test results are

assumed to be false negative or false positives, the sensitivity was 82% and specificity was 79%, still achieving statistical significance.

Likelihood Ratios: The positive and negative likelihood ratios were calculated with 95% confidence limits. The positive likelihood ratio showed a strong increase in the probability of correctly classifying a patient who has OUD with an Elevated Genetic Risk test result, and the reverse was true for the negative likelihood ratio with showed a strong decrease in the probability of correctly classifying a patient who does not have OUD with a Non-Elevated Genetic Risk test result.

Table 19-9b: Likelihood Ratios with Two-Sided 95% Confidence Limits

Statistic	Negative Likelihood Ratio	Positive Likelihood Ratio
Estimate	0.22	3.98
95% Confidence Limits	(0.17%, 0.33%)	(3.26%, 6.87%)

NPV and PPV: This test is intended to be used as part of a clinical evaluation and risk assessment to assess genetic risk of developing OUD in opioid naïve patients for whom the healthcare provider is considering prescribing oral opioids for 4-30 days for the treatment of acute pain, such as patients scheduled to undergo a planned surgical procedure, and who consent to having the test performed. The negative and positive predictive values and the chance of developing OUD with a negative or positive result are shown in the table for a 1% and 5% prevalence rate of OUD (see table) and will vary by the prevalence rate of OUD. Therefore, all the test results should be interpreted with caution and in conjunction with a complete clinical evaluation and risk assessment of the patient. The National Survey on Drug Use and Health estimated that approximately 1% of respondents had OUD (SAMHSA, Center for Behavioral Health Statistics and Quality, National Survey on Drug Use and Health, 2021).

Prevalence Assumption	Negative Predictive Value	Approximate Chance of OUD with a Negative Test Result	Positive Predictive Value	Approximate Chance of OUD with a Positive Test Result
1%	**99.8%**	**1 in 456**	**3.9%**	**1 in 26**
5%	**98.9%**	**1 in 88**	**17.3%**	**1 in 6**

Of the 381 patients in the study, 162 (42.5%) were female and 219 (57.5%) were male. 351 patients (92.1%) self-identified as white and 91 patients (23.8%) self-identified as Hispanic. **As a condition of approval, FDA is requiring additional post-approval studies to assess device performance in racially and ethnically diverse populations**.

Limitations of the clinical study:

- The study population was comprised of 92.1% of patients who self-identified as white. Only 24 (out of 381) clinical study patients self-identified as any other race or ethnicity. The study population was not powered to assess device performance in any other sub-population.

- The clinical study population was enriched for OUD, of which 73% had severe OUD. Results of the study may not represent performance in patients with mild or moderate OUD.
- Information on the comorbidities of the clinical study patients is limited. Many SNPs detected by AvertD® may also be associated with various psychiatric conditions and the presence of these psychiatric conditions was not determined for most subjects in the clinical trial.
- OUD status of patients in the clinical study was evaluated at the time of enrollment. Performance of the device in patients who have not yet developed OUD has not yet been evaluated.

15 REFERENCES

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13. APPENDIX

Comparison Testing with Eppendorf Mastercycler X50s

The OUD (AvertD) test was run with human genomic DNA samples purified from blood and buccal specimens as well as human cell lines purchased from Coriell Research Institute using two different Eppendorf thermocyclers, the Mastercycler EP with an aluminum block and the Mastercycler X50s with a silver block. Thermocycling conditions for the Mastercycler X50s are summarized in Tables A1.

Table A1. Amplification Reaction Conditions for AvertD with the Mastercycler X50s

Step No.	Temperature °C	Time	No. of Cycles
1	94	2 min.	1
2	94	15 sec.	10x
	69-60(-1.0/cycle)	15 sec.	
3	94	15 sec.	30X
	59	15 sec.	
4	94	15 sec.	1
5	4	HOLD	1

Note: Run time, 1.00 hour; temperature mode, standard; block settings, silver 96. After each cycle in step 2 the temperature is decreased by 1.0°C.

Forty eight DNA samples were amplified with both thermocyclers and run on the INFINITI PLUS system. Among the 47 valid AvertD test results, a total of 705 AvertD analyte genotype calls were 100% in agreement on the INFINITI HTS as shown in Table A2. In conclusion, both the Mastercycler EP and the Mastercycler X50s thermocyclers gave equivalent genotype calls with the INFINITI PLUS system.

Table A2. AvertD Genotype Call Comparison of Mastercycler EP and Mastercycler X50s

INFINITI System	INFINITI PLUS	
Thermocycler	Mastercycler EP	X50s
Total Samples	48	48
FTQ Samples	1	1
Valid Samples	47	47
Valid samples with both Thermocyclers	47	
Total SNP Calls	705	
Correct SNP Calls	705	
% SNP Call Agreement	100% (705/705)	