AvertD™

Package Insert
Instructions For Use

For *In Vitro* Diagnostic Use

EM-24069 DRAFT AvertD™ Package Insert (09-01-2022)
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1 INTENDED USE

AvertD™ is a prescription, qualitative genotyping test used to detect and identify 15 clinically relevant genetic polymorphisms in genomic DNA isolated from buccal samples collected from adults. The 15 detected genetic polymorphisms are involved in the brain reward pathways that are associated with opioid use disorder (OUD) and identify patients who may be at increased genetic risk for OUD. Information from AvertD™ provides patients 18 years of age or older and healthcare providers with objective information to be used for informed decision-making prior to the first prescription of oral opioids for acute pain. The information from AvertD™ is intended to be used in combination with a clinical evaluation and assessment of the patient.

2. BACKGROUND INFORMATION

Fifteen percent of U.S. adults filled one or more opioid prescriptions in 2018 (CDC 2019), typically for pain relief. Opioids cause addiction in some people, even when used as prescribed, due to a poorly understood combination of factors (genetics, mental health disorders, socioeconomic characteristics, access to illicit drugs).

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

- Drug overdose is the leading cause of death for Americans under 50 and opioids account for most overdose deaths (Drug Policy Alliance 2020).
- Every day, more than 130 Americans die from opioid addiction (CDC. America's Drug Overdose Epidemic: Data to Action 2020).
- Opioid overdose causes approximately 185,000 Emergency Room Department visits annually for patients aged 15 and older (CDC 2017).
- Opioid misuse and abuse disproportionately impact young Americans, with adults aged 26-34 years old having the highest percentage of self-reported prescription pain reliever misuse and abuse (CDC 2019).
- 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. Genetics appear to play a significant role in determining a person's risk for developing OUD, perhaps contributing more than 50% to overall risk. AvertD™ is a precision medicine test that identifies genetic predisposition to OUD, which will allow more informed decision-making by patients and providers regarding the choice between opioids and non-opioid alternatives.

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 swab specimens. These polymorphisms are involved in the brain reward pathways and are associated with opioid use disorder (OUD). AvertD™ identifies patients who may be at increased genetic risk for 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 specimen collection using the INFINITI® Buccal Sample Collection Kit
b) DNA extraction from the buccal sample
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 specimen. This information is processed by an algorithm that determines risk for opioid dependency.

The AvertD™ test report includes the genotype calls (allelic variants) and the risk for opioid use disorder as “YES”, “NO”, or “N/A” when genetic risk cannot be determined.

4 DEVICE DESCRIPTION

AvertD™ utilizes proprietary film-based microarray technology for multiplex detection of the 15 genetic polymorphisms involved in the brain reward pathways and associated with opioid use disorder (OUD).

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. AvertD™ 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 only with human buccal swab specimens collected using the INFINITI Buccal Swab Collection Kit.
- Specimens should be stored at room temperature.
- All patient specimens 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.

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 abnormal 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 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, specimens 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 Microaray:	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 swab specimen, with sufficient purity, i.e., with the absorbance ratio $A_{260}/A_{280} \geq 1.2$ and DNA concentration ≥ 1ng/µl and up to 60 ng/ µl. 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® PipetteTips (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)
- 8-well flat strip caps (Genesee Scientific, Catalog # 22-623)
- Thermocycler (Eppendorf Mastercycler Pro with aluminum block recommended)
- Pipettors
- Mini Centrifuge
- Microfuge Tube Racks
- Vortex
- 0.2 ml Thin Wall Tubes for PCR
- 1.5 ml Microcentrifuge Tubes

10 REQUIREMENTS

AvertD™ is designed to process buccal specimen collected using the INFINITI® Buccal Collection Kit. Buccal samples should be extracted following the manufacturer's instructions for the DNA extraction kit. Samples that have been collected within ≤ 60 days 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™.

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 thermal cycler used should be regularly maintained and calibrated with an external temperature standard, according to the laboratory's regulatory and QC requirements.

12 ASSAY PROCEDURE

12.1 DNA Extraction

Follow the instructions provided with the DNA extraction kit used.

12.2 Amplification Reaction

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.

12.2.1 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.

12.2.2 Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the 48- well plate.

12.2.3 Add 2 µl of sample DNA to each well.

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

12.2.4 Place the 48-well plate, sealed with 8-well flat strip caps 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: 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). If using other thermocycler models, we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

12.3 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.

12.3.1 Prepare the enzymes 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^{0}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

12.3.2 Dispense 2 µl of the enzyme mixture per reaction.

12.4 Sample Loading - INFINITI PLUS

Load the assembled 48WP with the associated lid (Catalog # 11-0030-00) or clean (see instructions in the INFINITI PLUS Operations Manual) 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).

13 INTERPRETATION OF RESULTS

13.1 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

13.2 AvertD™ will determine and report the genotype for each target allelic variant and the genetic risk of opioid use disorder (OUD). The risk of OUD will be printed on the Results Report provided by the INFINITI PLUS as "YES" if the patient has an increased risk of OUD and "NO" if patient does not have an increased risk of OUD.

13.3 Very rarely there could be an "IND" reported for a SNP possibly due to a novel genotype or SNP (not included in the panel) interfering with the common genotype detection. When any of the SNPs is reported as "IND", the Risk for OUD will be reported as "N/A". The test should be repeated.

13.4 A "no call" will be reported if the signal is not qualified, i.e., RFU is low. When any of the SNPs is reported as "no call", the Risk for OUD will be reported as "N/A". The test should be repeated.

13.5 DNA controls should make expected genotype calls for each allelic variant. The NTCE control should give "no calls" and the Risk for OUD will be reported as "Not applicable". If controls do not perform as expected, the run is considered invalid and should be repeated.

13.6 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.

14 LIMITATIONS

Genetics and lifestyle play a significant role in a person's risk for OUD.

The results obtained from AvertD™ are based on the patient's genetics and should be used and interpreted only in the context of an overall clinical diagnosis. The genetic testing results from AvertD™ are intended for use solely by a qualified health care professional. Any diagnosis, counseling, or treatment should use these results in conjunction with other patient information, including clinical presentation, patient history, family history, patient demographics, and other test results. The test results should not be the sole determinant in diagnosing, counseling, or making prescribing decisions. Non-genetic factors contribute to the likelihood of OUD and must also be considered in evaluating the appropriateness of opioid therapy.

15 PERFORMANCE CHARACTERISTICS

15.1 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.

15.2 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, 17.5 ng/ul, 63 ng/ul, 1 ng/ul, 0.3 ng/ul, and 0.1 ng/ul of DNA. The samples tested included the genotypes for the 15 genes evaluated in AvertD™. The genotypes 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%.

15.2 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 of > 99.995%.

Table 15-2: 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%

Allelic Variants	Genotype	Accuracy of AvertD™	
		Number of Alleles with Concordance	Percentage of Alleles with Concordance
	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%
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%

Allelic Variants	Genotype	Accuracy of AvertD™	
		Number of Alleles with Concordance	Percentage of Alleles with Concordance
	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%%

15.3 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. The samples covered all 15 genes evaluated by AvertD™. 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. 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 15-3** provide a summary of the Reproducibility Study results.

Table 15-3: 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%

Analytes	Samples Tested	Samples with Invalid Tests	Samples with Valid Results	Valid Samples with Discordant Calls	Valid Samples with Concordant Calls	Percent Concordant Calls
ABCB1	725	30	695	0	695	100.00%
Total	**10,875**	**450**	**10,425**	**0**	**10,425**	**100.00%**

15.4 Interfering Substances – Endogenous and Exogenous Substances

A study was conducted to evaluate the effect of potential endogenous and exogenous interfering substances on the performance of AvertD™. Buccal swab samples collected from individuals who have been directly exposed to the potential exogenous interferents were tested using AvertD™. Direct exposure to endogenous substances was not possible. Therefore, 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 the AvertD™ was observed for any of the tested substances, which include: antiseptic mouthwash, toothpaste, baking soda, cough syrup, cranberry juice, table salt, sugar, meat, chewing gum, hard candy, cigarettes, coffee and whole blood.

15.5 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.

15.6 Clinical Performance

The ability of AvertD™ assay to discriminate patients at higher genetic risk for developing OUD from patients with lower genetic risk was evaluated using buccal swab specimens collected from consenting subjects in a multicenter US clinical study trial. This clinical study was a multi-center, longitudinal study of subjects with a history of exposure to prescription oral opioids. For each subject, an assessment for OUD occurred at least 12 months following prescription oral opioid use, to allow sufficient time for OUD to develop. Each subject's confirmed OUD status was compared to the presence or absence of a genetic predisposition for OUD as determined by AvertD™.

385 subjects were randomly selected by a statistician from 10 clinical sites which covered a wide geographic distribution to be representative of the intended use population. All subjects were evaluated for OUD using a clinical evaluation. One central laboratory tested all study specimens, which contained study subject ID as the only identifier. The laboratory personnel (including laboratory technicians, supervisors and medical director) were masked to subject source, subject demographics, and subject clinical information including OUD status. All investigators and subjects were masked to the test results.

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

Sensitivity and Specificity: Overall AvertD™ had a sensitivity of 82.8% and specificity of 79.2% (see **Table 15-6a**). The results were statistically significant meeting the pre-specified performance goals (p value < 0.0001).

Table 15-6a: 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%

A sensitivity analysis was performed for the 4 subjects without a test result. In the sensitivity analysis, 1 of the 4 subjects was OUD positive and an imputed as a negative test result (assuming this is a false negative) and 3 subjects 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.

A series of sensitivity analyses was performed to determine whether gender, age, length of follow-up from opioid exposure, race or ethnicity affected sensitivity or specified. No statistically significant differences were observed for any of the variables, demonstrating robust test performance in all tested subgroups (see **Tables 15-6b** through **15-6e**).

Table 15-6b: Sensitivity and Specificity by Age Group and Sex

Sex	Age Group	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
Female	18-34	25	5	5	22	57	81.48	83.33%
Female	35-49	22	4	3	21	50	87.50%	84.62%
Female	50-64	23	6	1	10	40	90.91%	79.31%
Female	65+	5	6	1	3	15	75.00%	45.45%
Female	Total	75	21	10	56	162	84.85%	78.13%
Male	18-34	26	6	8	39	79	82.98%	81.25%
Male	35-49	29	6	6	31	72	83.78%	82.86%
Male	50-64	17	5	4	10	36	71.43%	77.27%
Male	65+	17	5	2	8	32	80.00%	77.27%
Male	Total	89	22	20	88	219	81.48%	80.18%
Both Sex	18-34	51	11	13	61	136	82.43%	82.26%
Both Sex	35-49	51	10	9	52	122	85.25%	83.61%
Both Sex	50-64	40	11	5	20	76	80.00%	78.43%
Both Sex	65+	22	11	3	11	47	78.57%	66.67%

Sex	Age Group	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
Both Sex	Grand Total	164	43	30	144	381	82.76%	79.23%
Sensitivity across age groups within females: Two-sided exact Kruskal-Wallis test p-value 0.81. Specificity across age groups within females: Two-sided exact Kruskal-Wallis test p-value 0.048. Sensitivity across age groups within males: Two-sided exact Kruskal-Wallis test p-value 0.77. Specificity across age groups within males: Two-sided exact Kruskal-Wallis test p-value 0.94. Sensitivity across age groups for both sexes combined: Two-sided exact Kruskal-Wallis test p-value 0.90. Specificity across age groups for both sexes combined: Two-sided exact Kruskal-Wallis test p-value 0.24. Sensitivity across females and males: Two-sided Fisher's exact test p-value 0.68. Specificity across females and males: Two-sided Fisher's exact test p-value 0.73.								

Table 15-6c: Sensitivity, Specificity by Length of Follow-up from Oral Opioid Index Exposure

Follow-up Group (years)	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
1-3	47	13	5	19	84	79.17%	78.33%
4+	117	30	25	125	297	83.33%	79.59%
Total	164	43	30	144	381	82.76%	79.23%
Sensitivity across follow-up from index opioid exposure categories: Two-sided Fisher's exact test p-value 0.57. Specificity across follow-up from index opioid exposure categories: Two-sided Fisher's exact test p-value 0.85.							

Table 15-6d: Sensitivity and Specificity by Race

Race	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
White	155	39	30	127	351	80.89%	79.90%
Non-white	9	3	0	12	24	100.00%	75.00%
Total	164	43	30	144	381	82.76%	79.23%
Sensitivity across race categories: Two-sided Fisher's exact test p-value 0.13. Specificity across race categories: Two-sided Fisher's exact test p-value 0.71.							

Table 15-6e: Sensitivity and Specificity by Ethnicity

Ethnicity	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
Hispanic	47	19	2	22	90	91.67%	71.21%
Non-Hispanic	117	24	28	116	285	80.56%	82.98%
Total	164	43	30	144	381	82.76%	79.23%
Sensitivity across ethnicity: Two-sided Fisher's exact test p-value 0.26. Specificity across ethnicity: Two-sided Fisher's exact test p-value 0.066.							

The study population for AvertD™ was designed to reflect the racial and ethnic distributions of its intended use population; i.e., patients in the U.S. who receive prescription oral opioids as part of their medical care. Although no differences in racial and ethnic groups were seen, some racial and ethnic groups in the study population were small and adequately powered statistical analysis to determine any differences in test performance could not be performed.

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 having OUD with a positive test result, and the reverse was true for the negative likelihood ratio with showed a strong decrease in the probability of having OUD with a negative test result.

Table 15-6f: 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%)

A series of sensitivity analyses was performed to determine whether gender, age, length of follow-up from opioid exposure, race or ethnicity affected the positive and negative likelihood ratios. No significant differences were observed for any of the variables as evidenced by the overlapping 95% confidence levels for all groups, demonstrating robust test performance in all tested subgroups.

16 REFERENCES

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