AutoGenomics

LifeKit® Predict (96)

Package Insert

Instructions For Use

IVD For *In Vitro* Diagnostic Use

℞ only Prescription Use Only

Single Use. Not for Reuse

CE

For Export Only

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1. INTENDED USE

LifeKit Predict (96) 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 LifeKit Predict (96) provides patients and healthcare providers with objective information for informed decision making regarding the use of oral opioids for acute pain relief. The information from LifeKit Predict (96) is intended to be used in combination with a clinical evaluation and assessment of the patient.

2. BACKGROUND INFORMATION

More than 116 million people worldwide are struggling with chronic pain and most require prescription drugs. When we give an opioid for pain relief, there is a continuum of responses, from good analgesia and improvement in function, to poor analgesia, to tolerance, to physical dependence, and to addiction.

Understanding of the genetics contributing to susceptibility to opiate addiction is crucial for the identification of potential novel therapeutic targets. Opioid misuse and addiction are an ongoing and rapidly evolving public health crisis, requiring innovative scientific solutions.

Genetic factors play a key role in opioid prescription addiction but are generally not evaluated in clinical practice. Currently, there is no objective way for practitioners to identify pain patients in medical management who are at risk to abuse or become addicted to prescribed medication or to identify those pain patients who will require high dosages or an unusual regimen of medication.

Effectively addressing this public health crisis will require many different interventions, including more selective use of opioids for acute pain relief. Genetics play a significant role in determining a person's risk for developing opioid use disorder (OUD), perhaps contributing more than 50% to overall risk.

Based on literature review in the area of pain management and addiction, we have developed LifeKit Predict designed to detect and identify genes involved in the mesolimbic dopamine pathway. These genes have been implicated in a number of behavioral phenomena including addiction, mental depression, and psychiatric disorders.

LifeKit Predict is a precision medicine test identifies genetic predisposition to OUD, which will allow more informed decision-making by healthcare providers and patients regarding the choice between opioids and non-opioid alternatives. By identifying the genetic risk and the most effective analgesic for an individual patient, the clinician could potentially improve the efficacy of the pain management and decrease the risk of iatrogenically-induced overdose, addiction and even death.

3 TEST PRINCIPLE/ASSAY OVERVIEW

LifeKit Predict (96) utilizes hybridization capture array with automated detection of multiplex PCR products. LifeKit Predict (96) 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). LifeKit Predict (96) identifies patients who may be at increased genetic risk for OUD.

The following are the 15 genetic polymorphisms detected by LifeKit Predict (96).

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

LifeKit Predict (96) 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
i) OUD risk assessment

Instrumentation for LifeKit Predict (96) is the INFINITI® High Throughput System (HTS). The HTS is CE-marked.

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 LifeKit Predict (96) 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

LifeKit Predict (96) utilizes AutoGenomics' 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).

LifeKit Predict is comprised of the following:

- BioFilmChip® Microarray
- Primer Extension Reagent
- 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 microarray chips are packaged in a tube, 48 chips to a tube. Two tubes are provided in the finished package.

The **Primer Extension Mix** is packaged in vials, each contains 0.5ml of the PE Mix. Four vials are packaged in the finished package.

The **Amplification Mix** provides the reagent for the PCR amplification of the DNA sample. The amplification mix is packaged in vials, each vial contains 0.5ml of the Amp Mix. Four vials are packaged in the finished package.

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 HTS, which performs the assay.

The **HTS** is a multiplexing microarray platform that integrates hybridization, stringency, and detection for the analysis of DNA. The HTS is comprised of the Incubator, the Processor, and the ACE Reader. The Incubator incubates microarray chips used in the assay. The Processor automates washing of the microarrays. The ACE Reader measures fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays, and reports assay results.

LifeKit Predict (96) 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 HTS are provided in EM-21348 INFINITI HTS 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 AutoGenomics 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).
- After adding SAP/EXO to the PCR product, it must be used immediately.

INFINITI HTS

- **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°C to 8°C)
PE Mix:	12 months Refrigerated (2°C to 8°C)
Amplification Mix:	12 months Frozen (-30°C to -15°C)

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

7 MATERIALS PROVIDED (SUFFICIENT FOR 96 TESTS)

- Product Number 03-5540-02 Microarray Tube, LifeKit Predict (96):
  2 tubes per package
  48 microarray chips per tube

- Product Number 03-6540-02 PE Mix, LifeKit Predict (96):
  4 vials per package
  0.5ml per vial

  The Primer Extension Mix contains:
  Extension Reaction Buffer
  Labeled-dCTP
  dNTPs
  Allele Specific Primers

- Product Number 03-7540-02 Amp Mix, LifeKit Predict (96):
  4 vials per package
  0.5ml per vial

  The Amplification Mix contains:
  Multiplex Primer Mix
  dNTPs
  PCR Buffer

- Product Number 12-0360-00: INFINITI Hybridization Buffer
- Product Number 12-0380-00: INFINITI Wash Buffer

8 REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - LifeKit Predict (96) 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 $\geq$ 1ng/µl. Any DNA extraction method that meets this specification may be used. Contact AutoGenomics for further information.
- Titanium Taq DNA Polymerase (Clontech Catalog # 639209)
- Shrimp Alkaline Phosphatase (SAP, Thermo Fsher, Catalog # 783905000UN)
- Exonuclease I (Exo I, Thermo Fsher, Catalog # EN0582)
- Distilled water (DNAse and RNAse free)

9 EQUIPMENT

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

- INFINITI® ACE Reader (Catalog # 10-0030-99)
- INFINITI® Processor (Catalog # 10-0060-99)
- INFINITI® Incubator (Catalog # 10-0070-99)
- INFINITI® 96-Well Chip Plate, (Catalog # 11-0130-00)
- INFINITI® Silicon Chip Plate Mat, (Catalog # 11-0160-00)
- Thermocycler (Eppendorf Mastercycler PRO with aluminum block recommended)
- 96-well PCR Plate (Eppendorf twin.tec PCR 96, PN 951020303 or 0030133404;VWR, Catalog # 47744-12 or -116) Make sure that the lids match the PCR plates (come from same vendor)
- PCR Adhesive Sealing Film, 3 ea. (for Eppendorf plates, PN 30127781, for VWR plates VWR Catalog # 60941-076). Make sure that the plates match (come from same vendor)
- Plate Spinner (Labnet MPS1000 is recommended)
- Multi well flat strip caps (match plates)
- Pipettors (Single and 8-channel pipettors are recommended)

- Reagent Reservoir/trough
- Mini Centrifuge
- 1.5 mL Micro-centrifuge Tubes (molecular grade)
- Microfuge Tube Racks
- Vortex Mixer
- Cold Racks

10 REQUIREMENTS

LifeKit Predict (96) 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. The purified DNA should be at a concentration of ≥1 ng/µl with an absorbance ratio A_{260}/A_{280} ≥1.2.

11 QUALITY CONTROL

A known positive control (heterozygous and/or homozygous samples), 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 AutoGenomics for recommendations on sources of well characterized DNA samples.

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 multi well plate.

Note: For optimal processing on HTS, it is recommended to process samples in multiples of 8.

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 multi-well plate in a thermocycler. Make sure to seal the multi-well plate with the recommended PCR film from Eppendorf to avoid sample evaporation in the wells at the edges of the plate. 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. If using other thermocycler models, we recommend adjusting the ramp rate 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.

12.3.3 Seal the multi-well plate, briefly vortex and place the multi well plate in a thermocycler. Immediately commence the cleanup reaction using the following program:

Step No.	Temperature °C	Time	No. of cycles
1	37	60 min.	1
2	95	20 min.	1
3	4	Hold	Hold

***Note*:** It is recommended to use an Eppendorf Mastercycler Pro with an aluminum block. The ramp rate for Eppendorf Mastercycler Pro should be set at 100% and the process should take approximately 1 hour and 30 min (± 5 min).

12.4 Primer Extension Reaction

Note: To avoid contamination, a separate area is recommended for assembly of the Primer Extension reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach. Filter tips and gloves must be used when handling specimens and controls.

12.4.1 Add 20 µL of Primer Extension Mix to each well and mix thoroughly by pipetting up and down twice.

12.4.2 Seal the Sample Plate (now containing Primer Extension Reagents) once more with PCR Film, vortex carefully and quickly spin for 30 seconds using a plate spinner. Place the Sample Plate in a thermal cycler and immediately start the primer extension reaction using the following program.

Step No.	**Temperature °C**	**Time**	**Ramp Rate**	**No. of Cycles**
1	98	300 sec.	100%	1
2	57 94	20 sec. 20 sec.	53% 37%	40X
3	4	Hold	Hold	Hold

Note: It is recommended that an Eppendorf Mastercycler PRO with an aluminum block be used.
For step No. 1, the ramp rate must be set at 100%.
For step No. 2, the ramp rate for Eppendorf Mastercycler PRO should be set according to the instructions in the above chart. From 94^0C to 57^0C at 53% cooling rate, and from 57^0C to 94^0C at 37% heating rate.
The Primer Extension step should take 1 hour and 30 minutes ± 5.

12.5 Hybridization Reaction on the INFINITI® INCUBATOR

12.5.1 Addition of Hybridization Buffer 1 in 96-well Micro well plate containing samples.

12.5.1.1 Before pipetting the Hybridization buffer 1, it is better to create a Source Plate (Plate or trough containing the Hybridization buffer 1).

12.5.1.2 After removing the multi well plate from the thermocycler, add 80µl of Hybridization Buffer 1 to each well and mix thoroughly.

12.5.2 Assembling the 96-Well Chip Tray

12.5.2.1 Orient the Chip Tray such that the groove along the length of the Chip Plate is facing the operator.

12.5.2.2 Remove the black Chip Tray grid by unscrewing the thumbscrews on its surface. Set aside the grid.

12.5.2.3 Place into each seat a BioFilmChip oriented "arrow up" on the Chip Tray and matching the notches on the left side of the chip as shown in Figure 1.

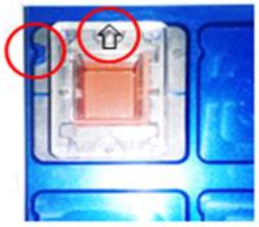

Figure 1

Note: If running less than a full plate of 96 microarrays, arrange the microarrays vertically (by column) in multiples of 8. Note that the HTS processes a minimum of 8 microarrays. If running less than 8 microarrays fill in the rest of the vertical column with used/old/dummy chips.

12.5.2.4 Replace the black Chip Tray grid and fasten by screwing back the thumbscrews on its surface. Take care to properly orient the grid such that the recessed screw inserts are facing up as shown in Figure 2.

Figure 2

12.5.3 Transferring Samples to Microarrays in the 96-Well Chip Plate

Transfer the entire volume (122µl) of each well to its designated microarray. See plate map below (Figure 3) for designations:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Figure 3 96-Microarray Chip Plate

12.5.4 Once all samples have been transferred, carefully place the white silicon Chip Plate mat over the Chip Plate containing the transferred samples.

12.5.4.1 Align the corner of the silicon mat to the Chip Plate and fit screw heads through the cutout holes on the silicon mat as shown in Figure 4 below.

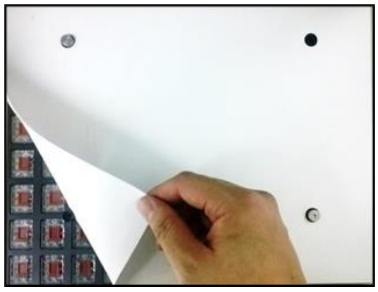

Figure 4

12.5.4.2 Take care not to dip or splash the liquid contents of the microarrays.

12.5.5 Carefully place the covered Chip Plate on one of the incubation shelves on the INFINITI INCUBATOR, and press the green "START" button next to the incubation slot to start the timer.

Note: The incubation will continue for 90 minutes at 40ºC.

12.5.6 Refer to the INFINITI HTS Operator's Manual for additional information.

Note: After incubation, the Chip Plate will be ready for washing on the INFINITI PROCESSOR.

12.6 Microarray Washing on the INFINITI® PROCESSOR

12.6.1 Operating the INFINITI Processor

12.6.1.1 Start up the INFINITI PROCESSOR operating system by clicking on the "Wash" icon. Once the system is ready a "System Ready" message will appear in the bottom message bar.

12.6.1.2 Ensure there is sufficient wash buffer in the tank connected to the INFINITI PROCESSOR.

12.6.1.3 Verify that the waste tank container connected to the INFINITI PROCESSOR is not full.

12.6.1.4 On the computer interface click on the "Clean" button to initiate a cleaning/purging sequence. The cleaning process stops automatically after 30 secs. Click on the "Clean" button once more to repeat the process.

12.6.2 Loading Chip Tray onto the INFINITI PROCESSOR

12.6.2.1 After hybridization is completed, carefully remove the silicon mat covering the Chip Plate.

12.6.2.2 Place the Chip Plate onto the deck of the INFINITI PROCESSOR for automated chip washing.

Note: There are 2 decks on the INFINITI PROCESSOR; if only 1 Chip Plate is to be washed, place the Chip Plate on the left most deck.

12.6.2.3 On the computer interface indicate the number of columns to be washed. Then click on the "Wash" button to begin the washing cycle.

12.6.2.4 Proceed to read the Microarrays on the INFINITI ACE Reader thereafter. Refer to the INFINITI HTS Operator's Manual for additional information.

Note: If a run needs to be stopped or aborted, the entire system must be POWERED DOWN before restarting.

12.6.2.5 Refer to the INFINITI® HTS operator's manual for additional information.

Note: After washing the Chip Plate will be ready for reading and analysis on the INFINITI ACE.

12.7 Reading Microarrays on the INFINITI® ACE

12.7.1 Start up the INFINITI ACE operating system by clicking on the "Reader" icon. Once the system is ready a "System Ready" message will appear in the bottom message bar.

12.7.2 Left click on the "Start" button to begin preparing for a run and an empty worklist worksheet will appear.

12.7.3 Generate a worklist for the samples to be processed.

12.7.3.1 The worklist can be created in two ways - Creating a pre-set work list through a text editor; or using the included dropdown menu from the Reader program.

12.7.3.2 Refer to INFINITI HTS operator's manual for additional information.

12.8 Accessing Results

To retrieve the results, click on the "Results.exe" icon located on the INFINITI ACE desktop.

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 LifeKit Predict (96) 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 ACE 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 Positive 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 HTS Operator's Manual.

14 LIMITATIONS

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

The results obtained from LifeKit Predict (96) 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 LifeKit Predict (96) 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. AutoGenomics is not responsible for any clinical decisions that are taken.

15 PERFORMANCE CHARACTERISTICS

15.1 Limits of Detection (analytical sensitivity)

The analytical sensitivity (Limit of Detection or LOD) of LifeKit Predict (96) was assessed by testing eight (8) samples at eight (8) serial dilutions (60, 30, 15, 7.5, 3, 1, 0.3, and 0.1 ng/µl DNA) and eight (8) replicates per DNA concentration. The samples were comprised of four (4) Coriell DNA and four (4) buccal DNA and included the genotypes for the 15 genes evaluated in LifeKit Predict (96). The genotypes were confirmed by bidirectional sequencing. A total of 512 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 at a concentration of 1ng/µl. At this lower limit, the percent correct call rate was 100.0% for all valid samples.

15.2 Assay Accuracy – Comparison Studies

270 buccal samples which were previously tested using LifeKit Predict (INFINITI PLUS) were tested using LifeKit Predict (96). The LifeKit Predict (INFINITI PLUS) results were confirmed by bi-directional sequencing. Three (3) HTS instruments and three (3) operators were involved in the comparison studies. 90 samples were tested on each HTS. Each sample was tested once.
Five (5) samples had no calls and were not included in the analysis, i.e., invalid samples. 264 of the 265 valid samples (99.6%) had HTS results which matched the INFINITI PLUS results. There was not enough sample to conduct additional investigation of the discordant result. 99.9% (3,973/3,975) of the genotype calls from the valid samples were correct.

Table 1 provides results of the comparison studies comparing LifeKit Predict using HTS to LifeKit Predict using INFINITI PLUS. Results using INFINITI PLUS were confirmed by bi-directional sequencing.

Table 1 Comparison Study Results

Instrument	Samples Tested	Initial Test					Final Test*				
		Samples with No Call/IND	Samples with Genotype Calls	Samples with Incorrect Genotype Calls	Samples with Correct Genotype Calls	% Samples with Correct Genotype Calls	Invalid Samples (with No Call/IND)	Valid Samples (with Genotype Calls)	Valid Samples with Incorrect Genotype Calls	Valid Samples with Correct Genotype Calls	% Valid Samples with Correct Genotype Calls
1	90	7	83	1	82	98.8%	5	85	1	84	98.8%
2	90	0	90	0	90	100%	0	90	0	90	100%
3	90	1	89	0	89	100%	0	90	0	90	100%
Total	270	8	262	1**	261	99.6%	5	265	1**	264	99.6%

* After repeats per protocol
** No sample remained to conduct additional investigation

15.3 Reproducibility Studies

The reproducibility studies involved three (3) HTS instruments and three (3) operators. The reproducibility sample panel consisted of 12 samples: four (4) Coriell DNA and eight (8) buccal specimens. To reduce variability due to extraction method, one extraction method was used, and the extracted DNA was aliquoted to the three (3) operators. In addition, one lot of LifeKit Predict (96) reagents was used. Each operator tested the DNA samples in triplicate on five (5) non-consecutive days.

The 36 DNA samples (12 x 3) and two (2) NTCE controls were positioned in the 96-well-plate following specific configurations. There were five (5) configurations, one per day. The same configurations were run by the three (3) operators.

The LifeKit Predict (96) results were compared to the expected genotypes obtained by bi-directional sequencing. A total of 540 tests were run (180 tests per operator). 539 of the 540 tests (99.8%) gave correct calls. The one test that failed, Sample ID #7 tested by operator 3 on day 5, gave an IND call for one analyte (GABA). All genotype calls made were 100% correct.

Table 2 provides a summary of the Reproducibility Study results for the genotypes tested.

Table 2: Reproducibility Study Results

Analytes	# Tests	No calls/IND	Genotype calls	Incorrect calls	Correct calls	% Correct calls
5-HTR2A	540	0	540	0	540	100%
COMT	540	0	540	0	540	100%
DRD1	540	0	540	0	540	100%
DRD2	540	0	540	0	540	100%
DRD4	540	0	540	0	540	100%
DAT1	540	0	540	0	540	100%
DBH	540	0	540	0	540	100%
MTHFR	540	0	540	0	540	100%
OPRK1	540	0	540	0	540	100%
GABA	540	1	539	0	539	100%
OPRM1	540	0	540	0	540	100%
MUOR	540	0	540	0	540	100%
GAL	540	0	540	0	540	100%
DOR	540	0	540	0	540	100%
ABCB1	540	0	540	0	540	100%
Total	**8,100**	**1**	**8,099**	0	**8,099**	100%

15.4 Interfering Substances – Endogenous and Exogenous Substances

In a study conducted to evaluate the effect of potential endogenous and exogenous interfering substances on the performance of LifeKit Predict, buccal swab samples collected from individuals who have been directly exposed to the potential exogenous interferents were tested using LifeKit Predict.

No interference with the LifeKit Predict 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.

15.5 Sample Carry-Over

The sample carry over studies involved four (4) Coriell DNA as samples for the study. Dilutions for each sample were prepared at 60ng/µl, 30ng/µl and 3ng/µl DNA. To evaluate for contamination due to sample carry-over, DNA samples and NTCE samples were set-up in the 96 well-plate to ensure that every position on the plate is addressed. Genotype calls were compared to the expected Coriell genotype calls. All samples gave the expected genotype calls.

The sample carry-over studies demonstrated that there is no contamination due to sample carry over when using the HTS for the LifeKit Predict test.

15.6 Clinical Performance

The ability of LifeKit Predict 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 LifeKit Predict.

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 with clinical standard practice or by a validated and widely-used research instrument – the Mini International Neuropsychiatric Interview, Version 7.0.2 (MINI). All prospective diagnoses were established by an expert consensus panel of board-certified physicians specialized in addiction medicine. The expert panel members were masked from the LifeKit Predict results, as well as to each other's diagnosis determination. Clinical performance of the LifeKit Predict assay was then evaluated. 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 in the Study Analysis Population, LifeKit Test results were available for 381 (99%). Test results were not available for 4 subjects due to inadequate DNA extraction from the buccal specimen. The distribution of these subjects by OUD diagnosis and Test Result appears in Table 3.

Table 3: Distribution of OUD Diagnosis and Test Results

		OUD Diagnosis		Total
		-	+	
LifeKit Predict	-	163	31	194
	+	41	146	187

Sensitivity and Specificity: Overall the LifeKit Predict had a sensitivity of 82.5% and specificity of 79.9% (see **Table 4**). The results were statistically significant meeting the pre-specified performance goals (p value < 0.0001).

Table 4: Sensitivity and Specificity of LifeKit Predict

		OUD Diagnosis		Total
		-	+	
LifeKit Predict	-	163	31	194
	+	41	146	187
		204	177	381
Sensitivity=100*(146/177) = 82.49 (76.07, 87.78) Specificity=100*(163/204) = 79.90 (73.74, 85.17)				

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 5** through **8**).

Table 5: 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
	35-49	21	4	4	21	50	84.00	84.00
	50-64	23	6	1	10	40	90.91	79.31
	65+	5	5	1	4	15	80.00	50.00
Total		74	20	11	57	162	83.82	78.72
Males	18-34	26	6	8	39	79	82.98	81.25
	35-49	29	6	6	31	72	83.78	82.86
	50-64	17	5	4	10	36	71.43	77.27
	65+	17	4	2	9	32	81.82	80.95
Total		89	21	20	89	219	81.65	80.91
Both Sexes	18-34	51	11	13	61	136	82.43	82.25
	35-49	50	10	10	52	122	83.67	83.33
	50-64	40	11	5	20	76	80.00	78.43
	65+	22	9	3	13	31	81.25	70.97
Grand Total		163	41	31	146	381	82.49	79.90
Sensitivity Across Age Group within Females P=0.9269 by two-sided Kruskal Wallis exact test. Specificity Across Age Group within Females P=0.1293 by two-sided Kruskal Wallis exact test. Sensitivity Across Age Group within Males P=0.7632 by two-sided Kruskal Wallis exact test. Specificity Across Age Group within Males P=0.9731 by two-sided Kruskal Wallis exact test.								

Sensitivity Across Age Group for Sexes Separated P=0.9647 by two-sided Fisher-Freeman-Halton exact test.
Specificity Across Age Group for Sexes Separated P=0.5908 by two-sided Fisher-Freeman-Halton exact test.
Sensitivity Across Age Group for Sexes combined P=0.9896 by two-sided Kruskal Wallis exact test.
Specificity Across Age Group for Sexes combined P=0.5233 by two-sided Kruskal Wallis exact test.
Sensitivity Across Females and Males P=0.8395 by Fishers two-sided exact test.
Specificity Across Females and Males P=0.7284 by Fishers two-sided exact test.

Table 6: Sensitivity, Specificity by Length of Follow-up from Opioid Exposure

Follow-up Group (Yrs)	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
1-3	46	12	6	20	84	76.92	79.31
4+	117	29	25	126	297	83.44	80.13
Total	163	41	31	146	381	82.49	79.90
Sensitivity across Follow-up groups P=0.4999 by two-sided Kruskal-Wallis test. Specificity across Follow-up groups P>0.9999 by two-sided Kruskal-Wallis test.							

Table 7: Sensitivity and Specificity by Race

Race	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
White	154	37	31	129	351	80.63	80.63
Non-White	9	3	0	12	24	100.00	75.00
Total	163	40	31	140	375	82.49	79.90
Sensitivity across Race groups P=0.1272 by two-sided Fishers exact test. Specificity across Race groups P=0.7074 by two-sided Fishers exact test.							

Table 8: Sensitivity and Specificity by Ethnicity

Race	True Negative	False Positive	False Negative	True Positive	Total	Sensitivity	Specificity
Hispanic	46	17	3	24	90	88.89	73.02
Non-Hispanic	117	24	28	116	285	80.56	82.98
Total	163	41	31	140	375[a]	81.87	79.90
Sensitivity across Race groups P=0.4180 by two-sided Fishers exact test. Specificity across Race groups P=0.1297 by two-sided Fishers exact test.							

[a] Six study subjects did not report ethnicity and they were all true positive OUD cases.

Likelihood Ratios: The positive and negative likelihood ratios were calculated with 95% confidence limits. The positive likelihood ratio (4.1) 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 (0.21) with showed a strong decrease in the probability of having OUD with a negative test result. See **Table 9**.

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

Variable	Negative Likelihood Ratio	Positive Likelihood Ratio
Estimate	0.2192	4.104
95% Confidence Limits	0.1581- 0.3040	3.096 - 5.441

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.

Predictive Values: The negative predictive value (NPV) and positive predictive value (PPV) were determined from the study data. The NPV was 84.02 with 95% confidence limits of 78.09 to 88.88. The PPV and 95% confidence limits were 78.07 and 71.45 - 83.78.

The prevalence in this study was 0.4646 due to the enrichment process. The formulas for computing NPV and PPV as functions of sensitivity, specificity and prevalence provide a means to compute expected values for other prevalence values. **Table 10** provides the NPV and PPV for a range of prevalence that may be expected using the study observed sensitivity of 82.49% and specificity of 79.90%.

Table 10: NPV and PPV across a Range of Other Prevalence Values

Prevalence	1-Prevalence	NPV	Chance of OUD with a negative test result	PPV	Chance of OUD with a positive test result
0.01	0.99	0.9978	1 in 455	0.0398	1 in 25
0.02	0.98	0.9955	1 in 222	0.0773	1 in 13
0.03	0.97	0.9933	1 in 149	0.1126	1 in 8.9
0.04	0.96	0.9909	1 in 110	0.1460	1 in 6.8
0.05	0.95	0.9886	1 in 88	0.1776	1 in 5.6
0.10	0.90	0.9762	1 in 42	0.3132	1 in 3.2
0.15	0.85	0.9628	1 in 27	0.4200	1 in 2.38
0.20	0.80	0.9480	1 in 19	0.5064	1 in 1.97
0.25	0.75	0.9319	1 in 15	0.5777	1 in 1.73
0.30	0.70	0.9141	1 in 12	0.6375	1 in 1.57
0.35	0.65	0.8944	1 in 9.5	0.6885	1 in 1.45
0.40	0.60	0.8725	1 in 7.8	0.7323	1 in 1.37
0.45	0.55	0.8479	1 in 6.6	0.7705	1 in 1.3
0.50	0.50	0.8202	1 in 5.6	0.8041	1 in 1.24

16 REFERENCES

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