May 24, 2019

Akonni Biosystems Inc.
% Rita King, CEO
MethodSense, Inc.
1 Copley Parkway, Suite 410
Morrisville, NC 27560

Re: K183530
Trade/Device Name: TruDiagnosis System
Regulation Number: 21 CFR 862.3360
Regulation Name: Drug metabolizing enzyme genotyping system
Regulatory Class: Class II
Product Code: ODW, ODV, NSU
Dated: April 26, 2019
Received: May 1, 2019

Dear Rita King:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kellie B. Kelm, Ph.D.
Acting Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
k183530

Device Name
TruDiagnosis® System

Indications for Use *(Describe)*
The TruDiagnosis® System is an in vitro diagnostic device intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology. The TruDiagnosis® System consists of the TruDx® 2000 Imager, the TruArray® Warfarin Sensitivity Test Kit, and the ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block.

The TruDx® 2000 Imager is an instrument intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology.

The TruArray® Warfarin Sensitivity Test Kit is an in vitro diagnostic test for the detection and genotyping of the 2C9*2, 2C9*3 alleles of the cytochrome P450 (CYP450) 2C9 gene locus and Vitamin K epoxide reductase Cl, VKORCl, gene promoter polymorphism (-1639) from genomic DNA of human saliva samples collected using the Oragene® Dx Device (OGD-500) as an aid in the identification of patients at risk for increased warfarin sensitivity. The TruArray® Warfarin Sensitivity Test Kit is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Akonni Biosystems, Inc. (K183530)

This 510(k) Summary is in conformance with 21CFR 807.92

Submitter: Akonni Biosystems, Inc.
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Company Contact: Charles Daitch
CEO

Date Prepared: May 22, 2019

Device Name and Classification

Trade Name: TruDiagnosis® System
Common Name: TruDiagnosis® System
Classification: Class II
Regulation Number: 21 CFR §862.3360 Drug Metabolizing Enzyme Genotyping Systems
21 CFR §864.7750 Prothrombin time test
21 CFR §862.2570 Instrumentation for Clinical Multiplex Test Systems

Classification Panel: Toxicology (91), Hematology (81), Chemistry (75)
Product Code: ODW- Cytochrom P450 2C9 (CYP450 2C9) Drug Metabolizing Enzyme Genotyping System
ODV- Vitamin K epoxide reductase complex subunit 1 (VKORC1) Genotyping System
NSU- Instrumentation for Clinical Multiplex Test Systems

Predicate Device:

Predicate Device	
Trade Name	eSensor Warfarin Sensitivity Test and XT-8 Instrument
Common Name	eSensor Warfarin Sensitivity Test and XT-8 Instrument
510(k) Submitter / Holder	Osmetech Molecular Diagnostics
510(k) Number	K073720
Regulation Number	21CFR §862.3360 – Drug Metabolism Enzyme Genotyping Test

Predicate Device	
	21CFR §864.7750 – Prothrombin Time Test 21CFR §862.2570 – Instrument for Clinical Multiplex Test Systems
Classification Panel	Toxicology, Hematology, Chemistry
Product Code	ODW Cytochrome P450 2C9 (CYP450 2C9) Drug Metabolizing Enzyme Genotyping System ODV Vitamin K epoxide reductase complex subunit 1 (VKORC1) Genotyping System NSU Instrumentation for Clinical Multiplex Test Systems

Device Description
The TruDiagnosis® System is an in vitro diagnostic device intended for processing and genotyping multiple genes in a DNA sample utilizing on-slide Polymerase Chain Reaction (PCR) gel-drop microarray technology. The TruDiagnosis® System consists of the:

- Hardware: TruDx® 2000 Imager
- Software: TruSpot™ Software
- Test Kit: TruArray® Warfarin Sensitivity Test Kit
    - TruArray® Test Slide
    - TruPlex™ reagents
- Thermal Cycler: ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block

Hardware: Akonni's microarray imager (TruDx® 2000) is an instrument that consists of a high-intensity green light emitting diode (LED), custom optics, and a digital grayscale camera.

- The purpose of the TruDx® 2000 Imager is to capture a fluorescence image of the microarray after completing the test.
- The user inserts the TruArray® Test Slide into the TruDx® 2000 Imager and follows the on-screen prompts. The resulting microarray image is automatically analyzed and reported with the TruSpot™ Software.

Software: Akonni's TruSpot™ Software, integrated within the imager, locates and segments each fluorescently labeled microarray Gel-Element and reports signal-to-noise ratios (SNR). Assay results interpreted by TruSpot™ Software program are assigned a genotype and presented to the end user in a report format.

Test Kit: The TruArray® Warfarin Sensitivity Test Kit includes consumables and reagents necessary to perform multiplex on-slide PCR amplification, and fluorogenic target-specific microarray-based hybridization. Specifically:

- The TruArray® Test Slide (loaded with target DNA) undergoes thermal cycling via asymmetric amplification to enrich single stranded fluorescently labeled complimentary strands to capture probes printed on the microarray (asymmetric PCR and allele specific hybridization occur in the same chamber). After hybridization, arrays are washed and dried, and the user then inserts the microarray into the TruDx® 2000 Imager and follows the on-screen prompts.

The warfarin assay performed with the test kit is an in vitro diagnostic test for the detection and genotyping of the 2C9*2, 2C9*3 alleles of the cytochrome P450 (CYP450) 2C9 gene locus and Vitamin K epoxied reductase C1, VKORC1 3673, gene promotor polymorphism (-1639) mutations from genomic DNA of human saliva samples collected using the Oragene® Dx OGD-500 Device (K110701).

Thermal Cycler: The ProFlex™ PCR System with the ProFlex™ 2x Flat Sample Block, a component of the TruDiagnosis System, is an end-point thermal cycler, specifically designed for the amplification of nucleic acids using the Polymerase Chain Reaction (PCR) process. The user interface includes a touchscreen with a graphical display that shows the time, status, and temperature for each run. A touchscreen keypad allows you to enter information into fields on the display screen.

Indications for Use

The TruDiagnosis® System is an in vitro diagnostic device intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology. The TruDiagnosis® System consists of the TruDx® 2000 Imager, the TruArray® Warfarin Sensitivity Test Kit, and the ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block.

The TruDx® 2000 Imager is an instrument intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology.

The TruArray® Warfarin Sensitivity Test Kit is an in vitro diagnostic test for the detection and genotyping of the 2C9*2, 2C9*3 alleles of the cytochrome P450 (CYP450) 2C9 gene locus and Vitamin K epoxide reductase Cl, VKORCl, gene promoter polymorphism (-1639) variants from genomic DNA of human saliva samples collected using the Oragene® Dx Device (OGD-500) as an aid in the identification of patients at risk for increased warfarin sensitivity. The TruArray® Warfarin Sensitivity Test Kit is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

Risk Analysis Method

The TruDiagnosis® System was assessed to determine the reasonably foreseeable sequences/combinations of events leading to hazardous situations and identify and document the harm to the users. A risk analysis was conducted in accordance with ISO 14971:2007 and ISO14971:2012, Medical devices -- Application of risk management to medical devices. Several risks were assessed, including, but not limited to, instrument and assay design, software malfunctions, hardware failure, security, and improper use.

Substantial Equivalence

The table below provides a detailed comparison of the TruDiagnosis System to the predicate device.

Detailed Comparison of the Subject and Predicate Devices:

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
Indications for Use	The TruDiagnosis® System is an in vitro diagnostic device intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology. The TruDiagnosis® System consists of the TruDx® 2000 Imager, the TruArray® Warfarin Sensitivity Test Kit, and the ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block. The TruDx® 2000 Imager is an instrument intended for processing and genotyping multiple genetic variants in a DNA sample utilizing on-slide PCR gel-drop microarray technology. The TruArray® Warfarin Sensitivity Test Kit is an in vitro diagnostic test for the detection and genotyping of the 2C9*2, 2C9*3 alleles of the cytochrome P450 (CYP450) 2C9 gene locus and Vitamin K epoxide reductase Cl, VKORCl, gene promoter polymorphism (-1639) variants from genomic DNA of human saliva samples collected using the Oragene® Dx Device (OGD-500) as an aid in the identification of patients at risk for increased warfarin sensitivity. The TruArray® Warfarin Sensitivity Test Kit is a qualitative assay for use in	Same	The TruDiagnosis System and the eSensor Warfarin Sensitivity Test and XT-8 System have the same indications for use.

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
	clinical laboratories upon prescription by the attending physician.		
Type of Use	Prescription Use	Prescription Use	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Technology	Fluorescence detection based gel-drop microarray	Electrochemical detection based microarray	The TruDiagnosis System is equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System. Both systems employ the detection of labeled probes on a microarray. The only difference is that the TruDiagnosis System uses a fluorescent labeled probe for fluorescence detection, and the eSensor system uses a ferrocene labeled probe for electrochemical detection. This difference in signal transduction detection method does not does not affect the intended use or the safety and effectiveness of the device.
Detection Chemistry	Fluorogenic target-specific microarray-based hybridization. Signal detection using fluorescence.	The signal probe contains electrochemically active ferrocene labels. Hybridization of the three-member complex at the electrode surface and subsequent application of an excitation voltage causes the ferrous ion in each ferrocene group to undergo cyclic oxidation and reduction at its characteristic redox potential, leading to loss or gain of an	The TruDiagnosis System is different from the eSensor Warfarin Sensitivity Test and XT-8 System. This difference does not affect the intended use or the safety and effectiveness of the device.

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
		electron, and the generation of an alternating current at the electrode surface that is measured using voltammetry.	
Signal Transduction	Fluorescence	Electrical current, voltammetry	The signal transduction of the TruDiagnosis System is different from the signal transduction of the eSensor Warfarin Sensitivity Test and XT-8 System. This difference does not affect the intended use or the safety and effectiveness of the device.
Test Type	Qualitative genetic test for single nucleotide polymorphism detection.	Qualitative genetic test for single nucleotide polymorphism detection.	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Genes and SNPs	• CYP4502C9*2 (430C>T) • CYP4502C9*3 (1075A>C) • VKORC1 (-1639G>A)	• CYP2C9*2 (430C>T) • CYP2C9*3 (1075A>C) • VKORC1 (-1639G>A)	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Microarray-based genotyping test for simultaneous detection (multiplex system) of DNA sequences	Yes	Yes	The TruDiagnosis System is equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System.
Microarray Device	TruArray Test Slide	eSensor Test Cartridge	The TruDiagnosis System's microarray device is equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System's microarray device.
Assay result output	Assay results interpreted by a software program and assigned a genotype presented to the end user in a report format.	Assay results are interpreted by a software program and are assigned a result that is presented to the end user in a report format.	The TruDiagnosis System is equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System.

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
Reference method	Bi-directional Sanger sequencing	Bi-directional Sanger sequencing	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Device Components	TruArray Test Slides, TruPlex Reagents, and the TruDx 2000 Imager	eSensor Test Cartridge, amplification and detection reagents, and the eSensor XT-8 System.	The components of the TruDiagnosis System are equivalent to the components of the eSensor Warfarin Sensitivity Test and XT-8 System.
Instrument	TruDx 2000 Imager	eSensor XT-8 System	The TruDiagnosis System includes an instrument equivalent to the instrument included in the eSensor Warfarin Sensitivity Test and XT-8 System.
Number of Simultaneous Tests	1 test	8 tests	The TruDx 2000 Imager can only process one test at a time, while the XT-8 System can process multiple tests simultaneously. This difference does not affect the intended use or the safety and effectiveness of the device.
Test Processing Time	1 test within 30 seconds	8 tests within 40 minutes	The TruDx 2000 Imager is different from the XT-8 System. This difference in test processing time does not affect the intended use or safety and effectiveness of the device.
User Interface	7” LCD touchscreen	15“ LCD touchscreen	The user interface of the TruDx Imager is equivalent to that of the eSensor XT-8 System. Both instruments include LCD touchscreen interfaces. The only difference is that the screen size of the TruDx 2000 Imager is smaller than that of the XT-8 System. However, this difference does not

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
			have an impact on the intended use or the safety and effectiveness of the device.
Power Requirements	Universal input, 90-264 Vac	100-240 Vac, 50/60 Hz, 600 W	The power requirements of TruDx 2000 Imager and the eSensor XT-8 System are equivalent. Both systems are designed to use AC line power.
Dimensions	Approximately 10.93” x 8.5” x 16”	18.11” x 15.75” x 16.14”	The TruDx 2000 Imager is smaller than the eSensor XT-8 System. . This difference in size does not have an impact on the intended use or the safety and effectiveness of the device.
Weight	Approximately 25 lbs	48.5 lbs	The TruDx 2000 Imager is lighter than the eSensor XT-8 System. This difference in weight does not have an impact on the intended use or the safety and effectiveness of the device.
Specimen type	Genomic DNA obtained from epithelial cells contained in human saliva samples collected using the Oragene Dx Device OGD-500.	Genomic DNA obtained from a human whole blood sample.	The specimen type of the TruDiagnosis System is different from the specimen type of the eSensor Warfarin Sensitivity Test and XT-8 System. This difference does not affect the safety and effectiveness or intended use of the device.
Number of SNPs	3	3	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.

Item	Subject Device TruDiagnosis® System	Predicate Device eSensor Warfarin Sensitivity Test and XT-8 System (K073720)	Comparison
Input gDNA	25 ng of genomic DNA (5 µL of 5 ng/µL genomic DNA sample)	10 ng of genomic DNA (5 µL of 2 ng/µL genomic DNA sample)	The TruDiagnosis System is different from the eSensor Warfarin Sensitivity Test and XT-8 System.
DNA Extraction	Qiagen method. QIAAMP DNA Mini Kit.	Manual ethanol extraction method	The TruDiagnosis System is different from the eSensor Warfarin Sensitivity Test and XT-8 System Test. This difference does not affect the safety and effectiveness or intended use of the device.
PCR Process	Closed amplicon. On-slide PCR occurs in the same chamber as the microarray. Asymmetric PCR is performed on-slide to eliminate the predicate's exonuclease step.	Open amplicon. Two steps occur off cartridge: 1) tube-based PCR and 2) exonuclease enzyme treatment to generate single stranded target DNA.	The PCR Process of the TruDiagnosis System is different from that of the eSensor Warfarin Sensitivity Test and XT-8 System. This difference does not affect the safety and effectiveness or intended use of the device.
Thermal Cycling Used	Yes	Yes	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Target of Detection	Single-nucleotide polymorphism	Single-nucleotide polymorphism	The TruDiagnosis System is identical to the eSensor Warfarin Sensitivity Test and XT-8 System.
Protocol Identification	Uses a barcode to store protocol and production information	Uses an internal memory chip (EEPROM) to store protocol and production information.	The TruDiagnosis System is equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System.

Substantial Equivalence Conclusions:
In conclusion, the TruDiagnosis System and the eSensor Warfarin Sensitivity Test and XT-8 System have the same indications for use related to aiding in the identification of patients at risk for increased warfarin sensitivity via genotyping multiple genetic variants in a DNA sample. The TruDx 2000 Imager and the eSensor XT-8 System have the same indications for use, in relation to the use of an instrument. The TruArray Warfarin Sensitivity Test Kit and the eSensor Warfarin Sensitivity Test have the same indications for use, related to Warfarin sensitivity genotyping of the *2 (430C>T) and * 3 (1075A>C) alleles of the cytochrome P450 (CYP450) 2C9 gene locus and the Vitamin K epoxide reductase C1 (VKORC1) gene promoter polymorphism (-1639G>A) from genomic DNA. The technological characteristics and testing demonstrate that the TruDiagnosis System is substantially equivalent to the eSensor Warfarin Sensitivity Test and XT-8 System and assures that the TruDiagnosis System is as safe and effective as the eSensor Warfarin Sensitivity Test and XT-8 System.

Performance Characteristics
A series of studies have been performed to validate the performance characteristics for the intended use of the TruDiagnosis® System. A total of five (5) performance characteristics studies have been conducted and two Stability Testing Studies were conducted to provide data on the kit configuration and expiry of the kit. A summary of each study's results is provided below.

Limit of Detection

An upper and lower limit of detection study was performed to assess the genotyping performance of the TruArray® Warfarin Sensitivity Test Kit across a range of genomic DNA input concentrations. In order to determine the lowest concentration of DNA at which this assay is accurate, serial dilutions (125, 25, 2.5, 0.25 ng) of three genomic DNA samples of different genotypes were assayed 40 times using the TruArray® Warfarin Sensitivity Test Kit. Two entire runs were repeated as a result of control failures and two additional runs were performed for individual test samples that gave a first-pass no-call result. The study was performed with reagents and tests from 5 lots of the TruArray® Warfarin Sensitivity Test Kit, using three TruDx® 2000 Imager instruments, and 7 operators over 12 testing days over the course of 20 total calendar days. A total of 362 samples were tested and all genotyping calls of the TruArray® Warfarin Sensitivity Test Kit were compared to their corollary bi-directional Sanger sequencing result. The recommended DNA input for the TruArray® Warfarin Sensitivity Test Kit is 25 ng at a concentration of 5 ng/uL.

Limit of Detection Study by Sample Genotype

	Genotype					First Pass Calls					Final Result [a]				
Donor	2C9 *2	2C9 *3	VKORC1	DNA Input (ng)	# Replicates Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
1	*1/*1	*1/*3	AA	125	40	40	0	0	100.0%	92.8%	40	0	0	100.0%	92.8%
1	*1/*1	*1/*3	AA	25	40	39	1	0	97.5%	88.7%	40	0	0	100.0%	92.8%
1	*1/*1	*1/*3	AA	2.5	40	39	1	0	97.5%	88.7%	40	0	0	100.0%	92.8%
1	*1/*1	*1/*3	AA	0.25 [c]	40	28	12	0	70.0%	56.0%	28	12	0	70.0%	56.0%
1	*1/*1	*1/*3	AA	2.5-125	120	118	2	0	98.3%	94.8%	120	0	0	100.0%	97.5%
2	*1/*2	*1/*3	AG	125	40	28	12 [d]	0	70.0%	56.0%	40	0	0	100.0%	92.8%
2	*1/*2	*1/*3	AG	25	40	40	0	0	100.0%	92.8%	40	0	0	100.0%	92.8%
2	*1/*2	*1/*3	AG	2.5	40	35	5	0	87.5%	75.5%	40	0	0	100.0%	92.8%
2	*1/*2	*1/*3	AG	0.25 [c]	40	15	22	3	37.5%	24.7%	15	22	3	37.5%	24.7%
2	*1/*2	*1/*3	AG	2.5-125	120	103	17	0	85.8%	79.5%	120	0	0	100.0%	97.5%
3	*2/*2	*1/*1	GG	125	42	25	17 [d]	0	59.5%	45.7%	40	0	0	100.0%	92.8%
3	*2/*2	*1/*1	GG	25	40	37	3	0	92.5%	81.7%	40	0	0	100.0%	92.8%
3	*2/*2	*1/*1	GG	2.5	40	36	4	0	90.0%	78.6%	40	0	0	100.0%	92.8%
3	*2/*2	*1/*1	GG	0.25 [c]	41	3	38	0	7.3%	2.0%	3	38	0	7.3%	2.0%
3	*2/*2	*1/*1	GG	2.5-125	122	98	24	0	80.3%	73.5%	120	0	0	100.0%	97.5%
	Total all 3 Samples			2.5-125	362	319	43 [d]	0	88.1%	85.0%	360	0	0	100.0%	99.2%

[a] Final Result represents a second repeat of a No Call. Incorrect Calls were not repeated.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

[c] Repeat runs were not performed.

[d] 2 runs had a negative control failure.

Limit of Detection Study by Loci

			First Pass Calls					Final Result [a]				
DNA Amount (ng)	Loci	Total Number of Calls	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
125	2C9*2	122	93	29 [c]	0	76.2%	69.0%	122	0	0	100.0%	97.6%
125	2C9*3	122	96	26 [c]	0	78.7%	71.7%	122	0	0	100.0%	97.6%
125	VKORC1	122	96	26 [c]	0	78.7%	71.7%	122	0	0	100.0%	97.6%
125	TOTAL	366	360	6 [c]	0	98.4%	96.8%	366	0	0	100.0%	99.2%
25	2C9*2	120	119	1	0	99.2%	96.1%	120	0	0	100.0%	97.5%
25	2C9*3	120	119	1	0	99.2%	96.1%	120	0	0	100.0%	97.5%
25	VKORC1	120	116	4	0	96.7%	92.5%	120	0	0	100.0%	97.5%
25	TOTAL	360	354	6	0	98.3%	96.7%	360	0	0	100.0%	99.2%
2.5	2C9*2	120	115	5	0	95.8%	91.4%	120	0	0	100.0%	97.5%
2.5	2C9*3	120	112	8	0	93.3%	88.3%	120	0	0	100.0%	97.5%
2.5	VKORC1	120	113	7	0	94.2%	89.3%	120	0	0	100.0%	97.5%
2.5	TOTAL	360	340	20	0	94.4%	92.0%	360	0	0	100.0%	99.2%
0.25 [c]	2C9*2	121	86	34	1	71.1%	63.5%	85	34	1	70.2%	62.7%
0.25 [c]	2C9*3	121	73	46	2	60.3%	52.5%	72	46	2	59.5%	51.6%
0.25 [c]	VKORC1	121	73	48	0	60.3%	52.5%	72	48	0	59.5%	51.6%
0.25 [c]	TOTAL	363	235	128	0	64.7%	60.4%	232	128	0	63.9%	59.6%

[a] Final Result represents a second repeat of a No Call. Incorrect Calls were not repeated.
[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.
[c] Repeat runs were not performed.
[d] 2 runs had a negative control failure.

Method Comparison

The method comparison evaluation was performed to assess the genotyping performance of the TruArray® Warfarin Sensitivity Test Kit as compared to bi-directional DNA sequencing across an extensive range of donor genotypes. Testing was conducted at one site (internal site). The test panel for the method comparison study consisted of 303 unique human genomic DNA samples isolated from saliva specimens collected using the Oragene®Dx OGD-500 device followed by DNA extraction using the Qiagen QIAamp DNA Mini Kit and genotype testing using the TruArray® Warfarin Sensitivity Test Kit.

The study was performed with four lots of the TruArray® Warfarin Sensitivity Test Kit, four TruDx® 2000 Imager instruments, and four operators over 14 testing days over the course of 30 total consecutive calendar days. The samples were typically run for their first-pass call in batches of 16 samples including 13 test samples, a negative extraction control, a positive PCR control and negative PCR control. An additional run was performed for tests that gave a first-pass no-call result.

Results are as summarized in the tables below. All genotyping calls of the TruArray® Warfarin Sensitivity Test Kit were compared to their corollary bi-directional sequencing result. For the first pass runs, a total of 303 donor samples were run resulting in 299 Correct Calls, 3 No Calls, and 1 Incorrect Calls. An investigation revealed that the incorrect call was the result of a sample mix-up. All 3 No Calls were resolved upon the second repeat runs. The Correct Call Rate for the first-pass runs is 98.7% and a 95% lower confidence bound of 97.0%. For the repeat runs (Final Result), the Correct Call Rate is 100.0% and a 95% lower confidence bound of 99.0%. The Final Results yielded 100% agreement between the TruArray® Warfarin Sensitivity Test Kit results and bi-directional DNA sequencing.

Agreement between TruArray® Warfarin Sensitivity Test Kit and Bi-directional DNA Sequencing (by Genotype):

Bi-directional Sequencing Sample Genotype				First Pass Calls					Final Result [a]				
2C9*2	2C9*3	VKORC1	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
*1/*1	*1/*1	AA	58	57	1	0	98.3%	92.1%	58	0	0	100.0%	95.0%
*1/*1	*1/*1	GA	79	79	0	0	100.0%	96.3%	79	0	0	100.0%	96.3%
*1/*1	*1/*1	GG	41	41	0	0	100.0%	93.0%	41	0	0	100.0%	93.0%
*1/*2	*1/*1	AA	8	8	0	0	100.0%	68.8%	8	0	0	100.0%	68.8%
*1/*2	*1/*1	GA	21	21	0	0	100.0%	86.7%	21	0	0	100.0%	86.7%
*1/*2	*1/*1	GG	30	29	1	0	96.7%	85.1%	30	0	0	100.0%	90.5%
*1/*1	*1/*3	AA	8	7	1	0	87.5%	52.9%	8	0	0	100.0%	68.8%
*1/*1	*1/*3	GA	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
*1/*1	*1/*3	GG	25	25	0	0	100.0%	88.7%	25	0	0	100.0%	88.7%
*2/*2	*1/*1	AA	1	1	0	0	100.0%	5.0%	1	0	0	100.0%	5.0%
*2/*2	*1/*1	GA	6	5	0	1 [c]	83.3%	41.8%	6	0	1 [c]	83.3%	41.8%
*2/*2	*1/*1	GG	3	3	0	0	100.0%	36.8%	3	0	0	100.0%	36.8%
*1/*1	*3/*3	GG	1	1	0	0	100.0%	5.0%	1	0	0	100.0%	5.0%
*1/*2	*1/*3	GA	6	6	0	0	100.0%	60.7%	6	0	0	100.0%	60.7%
*1/*2	*1/*3	GG	1	1	0	0	100.0%	5.0%	1	0	0	100.0%	5.0%
	Total		**303**	**299**	**3**	**1 [c]**	**98.7%**	**97.0%**	**302**	**0**	**1 [c]**	**99.7%**	**98.4%**

[a] Final Result represents a second repeat of a No Call. Incorrect Calls were not repeated.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

[c] One sample reported an incorrect call in the first run. Upon investigation, it was determined a sample mix up had occurred during accessioning. The first-pass call matched the bi-directional DNA sequencing result for the correctly accessioned samples. This sample was not re-run.

Agreement between TruArray® Warfarin Sensitivity Test Kit and Bi-directional DNA Sequencing (by Loci):

Bi-directional Sequencing Sample Genotype			First Pass Calls					Final Result [a]				
Loci	Genotype	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
2C9*2	wt/wt	227	227	0	0	100.0%	98.7%	227	0	0	100.0%	98.7%
2C9*2	wt/*2	66	66	0	0	100.0%	95.6%	66	0	0	100.0%	95.6%
2C9*2	*2/*2	10	10	0	0	100.0%	74.1%	10	0	0	100.0%	74.1%
2C9*3	wt/wt	247	247	0	0	100.0%	98.8%	247	0	0	100.0%	98.8%
2C9*3	wt/*3	55	54	1	0	98.2%	91.7%	55	0	0	100.0%	94.7%
2C9*3	*3/*3	1	1	0	0	100.0%	5.0%	1	0	0	100.0%	5.0%
VKORC1 (-1639)	GG	102	101	1	0	99.0%	95.4%	102	0	0	100.0%	97.1%
VKORC1 (-1639)	GA	126	125	0	1 [c]	99.2%	96.3%	125	0	1 [c]	99.2%	96.3%
VKORC1 (-1639)	AA	75	74	1	0	98.7%	93.8%	75	0	0	100.0%	96.1%

[a] Final Result represents a second repeat of a No Call. Incorrect Calls were not repeated.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

[c] One sample reported an incorrect call in the first run. Upon investigation, it was determined a sample mix up had occurred during accessioning. The first-pass call matched the bi-directional DNA sequencing result for the correctly accessioned samples. This sample was not re-run.

Interference Substances

Effect of Endogenous Interfering Substances:

Interfering substances including salivary α-amylase, hemoglobin, immunoglobulin A (IgA) and total protein were spiked into saliva samples in the amounts used by the predicate (GenMark, k110786). Saliva samples were collected using the OrageneDx OGD-500 device and extracted using the Qiagen QIAamp DSP DNA Mini Kit. Five donors provided saliva samples, each of which were divided into five aliquots and spiked with one of the four interfering substances. The fifth aliquot remained un-spiked and served as a control. Three extractions were performed on each spiked and un-spiked sample.

The study was performed with reagents and tests from 3 lots of the TruArray® Warfarin Sensitivity Test Kit, using two TruDx® 2000 Imager instruments, and two operators over 5 testing days over the course of 8 total calendar days.

All genotyping calls of the TruArray® Warfarin Sensitivity Test Kit were compared to their corollary bi-directional sequencing result. The first run for the study resulted in 3 No Calls which were resolved upon the repeat run. The Final Results yielded 100% agreement between the TruArray® Warfarin Sensitivity Test Kit results and bi-directional DNA sequencing for all test substances demonstrating no effect of any interfering substances on genotyping.

Endogenous Interference

			First Pass Calls					Final Result [a]				
Interference Condition	Interference Concentration	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
Control Total	N/A	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Amylase Total	311 - 341 U	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Hemoglobin Total	21.2 - 22.1 mg/mL	15	14	1	0	93.3%	72.1%	15	0	0	100.0%	81.9%
IgA Total	0.3 - 0.5 mg/mL	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Protein Total	2.1 - 2.7 mg/mL	15	13	2	0	86.7%	63.7%	15	0	0	100.0%	81.9%

[a] Final Result represents a second repeat of a No Call.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

Effect of Exogenous Interfering Substances:

Potentially interfering exogenous substances introduced into saliva samples through various activities (eating, drinking, chewing gum, mouthwash and smoking) were tested. Each activity group was composed of 5 donors who each provided 2 saliva samples - a baseline/control sample prior to the activity, and a sample collected 30 minutes after the activity. Samples were tested in triplicate. Saliva samples were collected using the OrageneDx OGD-500 device and extracted using the Qiagen QIAamp DSP DNA Mini Kit. A total of 150 samples were tested and all genotyping calls of the TruArray® Warfarin Sensitivity Test Kit were compared to their corollary bi-directional Sanger sequencing result.

Exogenous Interference

			First Pass Calls					Final Result [a]				
Activity	Time-point	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
Eating	Baseline	15	14	1	0	93.3%	72.1%	15	0	0	100.0%	81.9%
	30 min.	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Drinking	Baseline	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
	30 min.	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Chewing Gum	Baseline	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
	30 min.	15	13	2	0	86.7%	63.7%	15	0	0	100.0%	81.9%
Mouth Wash	Baseline	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
	30 min.	15	15	0	0	100.0%	81.9%	15	0	0	100.0%	81.9%
Smoking	Baseline	15	6	9 [c]	0	40.0%	19.0%	15	0	0	100.0%	81.9%
	30 min.	15	9	6 [c]	0	60.0%	36.0%	15	0	0	100.0%	81.9%
Total	Baseline	75	65	10	0	86.7%	78.4%	75	0	0	100.0%	96.1%
	30 min.	75	67	8	0	89.3%	81.6%	75	0	0	100.0%	96.1%

[a] Final Result represents a second repeat of a No Call.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

[b] 1 run in this set had a negative control failure. The run consisted of 9 pre-smoking calls and 5 post-smoking calls which were all Correct Calls when repeated.

Reproducibility

A reproducibility study was performed to assess the variability of the TruArray® Warfarin Sensitivity Test Kit between clinical study sites, operators, days, sample genotypes, instruments, and different lots of TruArray® Warfarin Sensitivity Test Kit. Six specific saliva samples, covering all genotypes detected by the TruArray® Warfarin Sensitivity Test Kit were used for this study. Saliva samples were collected using the OrageneDx OGD-500 device and extracted using the Qiagen QIAamp DSP DNA Mini Kit. The study was performed at 3 sites with 4 different lots of the TruArray® Warfarin Sensitivity Test Kit, using 4 TruDx™ 2000 Imager instruments, 4 ProFlex™ PCR Systems using the ProFlex™ 2x Flat Sample Block, 6 different genotype saliva samples, and 4 operators testing over 20 nonconsecutive. Site 1 (internal) had two operators and Site 2 and Site 3 each had one operator for a study total of four operators. Each operator processed a single sample (genotype) in triplicate and repeated over 5 non-consecutive days (15 tests per operator per genotype). Four operators generated 60 data points per genotype (4 operators x 15 tests per operator per genotype). 6 different sample genotypes generated 90 data points per operator (6 genotypes x 15 tests per operator per genotype). A total of 360 samples were tested and all genotyping calls of the TruArray® Warfarin Sensitivity Test Kit were compared to their corollary bi-directional Sanger sequencing result.

Reproducibility Study by Sample Genotype

Sequencing Genotype				First Pass Results					Final Results [a]				
2C9 *2	2C9 *3	VKORC1	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
*1/*1	*3/*3	GG	60	52	8	0	86.7%	77.2%	60	0	0	100.0%	95.1%
*1/*2	*1/*1	AA	60	53	7	0	88.3%	79.2%	60	0	0	100.0%	95.1%
*1/*1	*1/*1	GG	60	50	10	0	83.3%	73.4%	60	0	0	100.0%	95.1%
*1/*1	*1/*3	AA	60	50	10	0	83.3%	73.4%	60	0	0	100.0%	95.1%
*2/*2	*1/*1	GA	60	50	10	0	83.3%	73.4%	60	0	0	100.0%	95.1%
*1/*2	*1/*3	GA	60	48	12	0	80.0%	69.6%	60	0	0	100.0%	95.1%
Total			360	303	57	0	84.2%	80.7%	360	0	0	100.0%	99.2%

[a] Final Result represents up to four repeats of a No Call.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

Reproducibility Study by Site, Operator and Loci

				First Pass Results					Final Results [a]				
Site	Operator	Loci	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
1	1	2C9*2	90	78	12	0	86.7%	79.3%	90	0	0	100.0%	96.7%
		2C9*3	90	79	11	0	87.8%	80.6%	90	0	0	100.0%	96.7%
		VKORC1	90	79	11	0	87.8%	80.6%	90	0	0	100.0%	96.7%
1	2	2C9*2	90	82	8	0	91.1%	84.5%	90	0	0	100.0%	96.7%
		2C9*3	90	79	11	0	87.8%	80.6%	90	0	0	100.0%	96.7%
		VKORC1	90	80	10	0	88.9%	81.9%	90	0	0	100.0%	96.7%
2	3	2C9*2	90	71	19	0	78.9%	70.6%	90	0	0	100.0%	96.7%
		2C9*3	90	70	20	0	77.8%	69.4%	90	0	0	100.0%	96.7%
		VKORC1	90	71	19	0	78.9%	70.6%	90	0	0	100.0%	96.7%
3	4	2C9*2	90	80	10	0	88.9%	81.9%	90	0	0	100.0%	96.7%
		2C9*3	90	80	10	0	88.9%	81.9%	90	0	0	100.0%	96.7%
		VKORC1	90	78	12	0	86.7%	79.3%	90	0	0	100.0%	96.7%
Total	Loci	2C9*2	360	311	49	0	86.4%	83.1%	360	0	0	100.0%	99.2%
Total	Loci	2C9*3	360	308	52	0	85.6%	82.2%	360	0	0	100.0%	99.2%
Total	Loci	VKORC1	360	308	52	0	85.6%	82.2%	360	0	0	100.0%	99.2%
Total	SNPs		1080	927	153	0	85.8%	84.0%	1080	0	0	100.0%	99.7%

[a] Final Result represents up to four repeats of a No Call.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

Reproducibility Study by Production Lot and Loci

			First Pass Results					Final Results [a]				
Lot	Loci	# Samples Tested	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB	# Correct Calls	# No Calls	# Incorrect Calls	Correct Call Rate [b]	95% LCB
#1 5007/5008	2C9*2	72	71	1	0	98.6%	93.6%	72	0	0	100.0%	95.9%
	2C9*3	72	71	1	0	98.6%	93.6%	72	0	0	100.0%	95.9%
	VKORC1	72	70	2	0	97.2%	91.5%	72	0	0	100.0%	95.9%
#2 0011/0008	2C9*2	108	87	21	0	80.6%	73.2%	108	0	0	100.0%	97.3%
	2C9*3	108	87	21	0	80.6%	73.2%	108	0	0	100.0%	97.3%
	VKORC1	108	87	21	0	80.6%	73.2%	108	0	0	100.0%	97.3%
#3 8005/8004	2C9*2	108	98	10	0	90.7%	84.8%	108	0	0	100.0%	97.3%
	2C9*3	108	96	12	0	88.9%	82.6%	108	0	0	100.0%	97.3%
	VKORC1	108	96	12	0	88.9%	82.6%	108	0	0	100.0%	97.3%
#4 6010/0015	2C9*2	72	55	17	0	76.4%	66.7%	72	0	0	100.0%	95.9%
	2C9*3	72	54	18	0	75.0%	65.2%	72	0	0	100.0%	95.9%
	VKORC1	72	55	17	0	76.4%	66.7%	72	0	0	100.0%	95.9%
All Lots	2C9*2	360	311	49	0	86.4%	83.1%	360	0	0	100.0%	99.2%
	2C9*3	360	308	52	0	85.6%	82.2%	360	0	0	100.0%	99.2%
	VKORC1	360	308	52	0	85.6%	82.2%	360	0	0	100.0%	99.2%
SNPs		1080	927	153	0	85.8%	84.0%	1080	0	0	100.0%	99.7%

[a] Final Result represents up to four repeats of a No Call.

[b] The Correct Call rate is the proportion of Correct Calls from the total number of calls.

Kit Stability

Akonni conducted a Stability study to determine the expiration date of the TruArray® Warfarin Sensitivity Test Kit. Based on this 9 months of Stability testing, an expiry date of 6 months was determined for the TruArray® Warfarin Sensitivity Test Kit.

EMC and Safety Testing

Safety testing was performed on the TruDiagnosis® System in accordance with the following Standard:

- IEC 61010-1:2010 3rd edition: General Requirements for Basic Safety and Essential Performance
- EN 61326-1:2013 / EN 55011:2010 / EN 55022:2010: Electromagnetic Compatibility (EMC)
- IEC 60601-1-2:2014 Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility - Requirements and tests

TruDx® Imager and TruSpot™ Software Performance Testing

Verification testing for the TruDx® 2000 Imager was performed to confirm the documented Product Requirements. A total of eight (8) different verification tests were performed, as listed below.

- Mechanical Verification
- Electrical Verification
- Functional Verification
- Shipping and Packaging
- Environmental Verification
- Reliability Verification
- Hardware Interface Verification
- Performance Verification
- Software Verification

TruDx® Imager and TruSpot™ Software were validated in accordance with a Validation plan to ensure conformance with established performance criteria.

Conclusion

The 510(k) Pre-market Notification for the TruDiagnosis® System contains adequate information and data to determine that Akonni's TruDiagnosis® System is as safe and effective as the legally marketed predicate device(s).