510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

k183530

B. Purpose for Submission:

New Device

C. Measurand:

Genotype of Cytochrome P450 2C9 (CYP2C9) and Vitamin K Epoxide Reductase Complex Subunit 1 (VKORC1)

D. Type of Test:

Qualitative genotyping

E. Applicant:

Akonni Biosystems Inc.

F. Proprietary and Established Names:

TruDiagnosis® System

G. Regulatory Information:

Product Code	Regulation Name	Classification	Regulation Section	Panel
ODW	Drug metabolizing enzyme genotyping system	Class II	21 CFR 862.3360	Toxicology (91)
ODV	Prothrombin time test	Class II	21 CFR 864.7750	Hematology (81)
NSU	Instrumentation for clinical multiplex test systems	Class II	21 CFR 862.2570	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indication(s) for use below.

2. Indication(s) 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 C1, VKORC1, 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.

3. Special conditions for use statement(s):

   For prescription use only.

   The information provided from this test may supplement therapeutic decision-making and should only be used in conjunction with routine monitoring by a physician. Clinicians should use professional judgment in the interpretation of results from this type of test.

4. Special instrument requirements:

   TruDx® 2000 Imager
   ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block that is provided by Akonni with the TruDiagnosis® System
   Oragene® Dx collection device OGD-500
   Qiagen QIAamp DSP DNA Mini Kit

I. 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 following:

- Hardware: TruDx® 2000 Imager, ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block that is provided by Akonni with the TruDiagnosis® System
- Software: TruSpot™ Software
- Test Kit: TruArray® Warfarin Sensitivity Test Kit

Hardware: 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. 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.

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 the 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. The TruArray® Warfarin Sensitivity Test Kit consists of the TruPlex PCR Master Mix, TruPlex Warfarin Primer Mix, Formamide (deionized), TruArray Wash Buffer, and TruArray Warfarin Test Slide. The TruArray® Warfarin Sensitivity Test Kit is intended for use with DNA extracted from saliva collected from Oragene® Dx OGD-50 device using the Qiagen QIAamp DSP DNA Mini Kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   eSensor Warfarin Sensitivity Test and XT-8 Instrument

2. Predicate 510(k) number(s):

   k073720

3. Comparison with predicate:

Similarities		
Item	TruDiagnosis® System Candidate Device	eSensor Warfarin Sensitivity Test and eSensor XT-8 Instrument (k073720) Predicate Device
Indication(s) for use	Aid in the identification of patients at risk for increased warfarin sensitivity.	Same
Test type	Qualitative genetic test for single nucleotide polymorphism detection	Same
Gene/Mutations detected	CYP2C9*2 (430C>T) CYP2C9*3 (1075A>T) VKORC1 (-1639G>A)	Same

Differences		
Item	TruDiagnosis® System Candidate Device	eSensor Warfarin Sensitivity Test and eSensor XT-8 Instrument (k073720) Predicate Device
Detection Method	Fluorescence detection based gel-drop microarray	Electrochemical detection based microarray
Microarray substrate	Slide	Test cartridge
Sample Type	Genomic DNA from human saliva	Genomic DNA from human whole blood
Extraction	QIAamp DSP DNA Mini Kit	Multiple DNA extraction methods

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

The TruArray® Warfarin Sensitivity Test Kit and genomic DNA (gDNA) extracted from saliva collected using the OrageneDx OGD-500 and the Qiagen QIAamp DSP DNA Mini Kit are used to perform on-slide PCR and hybridization within the TruArray® Test Slide using the ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block followed by imaging on the TruDx® 2000 Imager.

Amplification reactions are prepared using the TruArray® Warfarin Sensitivity Test Kit components (TruPlex Master Mix, TruPlex Warfarin Primer Mix and Formamide (deionized)) and extracted gDNA. Amplification reactions are loaded into the inlet port on the TruArray® Test Slide.

PCR amplification and microarray hybridization occur simultaneously in the same reaction chamber in the TruArray® Warfarin Test Slide on the ProFlex™ PCR System using the ProFlex™ 2x Flat Sample Block provided by Akonni with the TruDiagnosis® System. DNA is amplified and hybridized using a specified thermal cycling protocol. After the amplification and hybridization process, the array is washed by piercing the inlet port with a pipettor tip and dispensing 1000 µL of the TruArray® Wash Buffer through the chamber. The excess PCR amplicons, reaction by-products, and the wash buffer are retained on-slide in the integrated waste chamber. The TruArray® Test Slide is placed in a 50 mL conical tube and centrifuged at 540 x g for 2 minutes in a non-refrigerated centrifuge to dry the array chamber prior to imaging.

The TruArray® Tests Slides are prepared using TruArray® microarray technology. The TruArray® is a spatially ordered array of three-dimensional hydrogel droplets, each droplet containing a unique capture probe. The hydrogel matrix is covalently attached to a glass substrate and oligonucleotide probes are covalently attached to the hydrogel scaffold. The purpose of the TruArray® is to specifically detect fluorescently labeled target amplicons via microarray-based hybridization.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility of the TruDiagnosis® System was evaluated at three sites (two external and one internal) over 5 days. Eight saliva samples from each of six donors covering all possible genotypes for all three alleles in the TruDiagnosis® System were collected using the Oragene® Dx OGD-500 collection device. Using saliva from two collection tubes per donor, DNA was extracted using the Qiagen QIAamp DSP DNA Mini Kit in triplicate for five non-consecutive days by each of four operators (15 replicates per operator per genotype) for a total of 60 replicates per donor/genotype. Four operators performed the reproducibility study across the three sites using four lots of TruArray® Warfarin Sensitivity Test Kit reagents and three TruDx® 2000 Imagers. Results from the TruDiagnosis® System were compared to the genotype for each sample as determined by bidirectional Sanger sequencing.

Donor/Sample	CYP2C9*2	CYP2C9*3	VKORC1 -1639
1	*1/*1	*3/*3	G/G
2	*2/*2	*1/*1	G/A
3	*1/*1	*1/*1	G/G
4	*1/*2	*1/*3	G/A
5	*1/*1	*1/*3	A/A
6	*1/*2	*1/*1	A/G

Results are summarized in the table below:

Genotype			# samples tested	First Run Results				Final Results[a]			
CYP 2C9*2	CYP 2C9*3	VKORC1 -1639		# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)	# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)
*1/*1	*3/*3	GG	60	52	8	0	86.7	60	0	0	100
*1/*2	*1/*1	AA	60	53	7	0	88.3	60	0	0	100
*1/*1	*1/*1	GG	60	50	10	0	83.3	60	0	0	100
*1/*1	*1/*3	AA	60	50	10	0	83.3	60	0	0	100
*2/*2	*1/*1	GA	60	50	10	0	83.3	60	0	0	100
*1/*2	*1/*3	GA	60	48	12	0	80	60	0	0	100
Total			360	303	57	0	84.2	360	0	0	100

[a]Final Results reflect up to four repeats of samples with no calls during the first run and subsequent runs
[b]Correct call rate = # correct calls / # of samples tested

Analysis of the data by operator and by lot was reviewed and supported there was no difference in precision between operators or between lots.

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls: Positive or negative controls are not included with this assay. The manufacturer has recommended that an external positive and negative control should be processed with each run of 16 test slides to assure the integrity of sample preparation, kit reagents, individual laboratory practices, and proper functioning of the system. The external positive control(s) should be 25 ng human gDNA of known *2, *3 and VKORC1 genotype; the external negative control should be a reagent blank (e.g., nuclease-free water). All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations.

Stability: The sponsor states in their labeling that the TruArray® Warfarin Sensitivity Test Kit components are stable for up to six months when stored as follows:

- TruPlex PCR Master Mix: -15°C to -25°C

- TruPlex Warfarin Primer Mix: -15°C to -25°C
- Formamide (deionized): -15°C to -25°C
- TruArray Wash Buffer: 17°C to 27°C
- TruArray Warfarin Test Slide: 2°C to 8°C

d. Detection limit:

An upper and lower limit of detection study was performed to assess the genotyping performance of the TruDiagnosis® System across a range of genomic DNA input concentrations. Serial dilutions (125, 25, 2.5, 0.25 ng) of three genomic DNA samples of different genotypes extracted using the Qiagen QIAamp DSP DNA Mini Kit from saliva samples collected using the Oragene® Dx OGD-500 device were assayed 40 times using the TruDiagnosis® System. Seven operators performed the testing over 12 days using five reagent lots of the TruArray® Warfarin Sensitivity Test Kit and three TruDx® 2000 Imagers. Results from the TruDiagnosis® System were compared to the genotype for each sample as determined by bidirectional Sanger sequencing. Results are as summarized in the table below.

The recommended DNA input for the TruDiagnosis® System is 25 ng at a concentration of 5 ng/µL. The package insert states:

- *"The recommended DNA input is 25 ng at a concentration of 5 ng/µL"*
- *"Failure to provide 25 ng purified gDNA may generate invalid test results. Samples with DNA concentrations less than 2.5 ng may generate incorrect results"*

e. Analytical specificity:

An evaluation of potential assay interferences was performed to assess the genotyping performance of the TruDiagnosis® System, when potential interferents are present in saliva samples.

<u>Endogenous Interferents</u>
Five donors provided saliva samples collected using the Oragene® Dx collection device OGD-500. Each saliva sample was divided into five aliquots and spiked with one of four potentially interfering substances. The fifth aliquot was not spiked with any potentially interfering substance and served as a control. Three extractions using the Qiagen QIAamp DSP DNA Mini Kit were performed on each sample.

The genotypes as determined by bidirectional Sanger sequencing for the five donors were as follows:

Donor/Sample	CYP2C9*2	CYP2C9*3	VKORC1 -1639
1	*1/*1	*3/*3	G/G
2	*1/*1	*1/*1	G/G
3	*1/*2	*1/*1	G/A
4	*1/*1	*1/*3	A/A
5	*2/*2	*1/*1	G/A

Results from the testing are summarized below. There was 100% agreement between the TruDiagnosis® System and bidirectional DNA sequencing for all test substances following a first run, or a second run for no calls observed during the first run, supporting that these interfering substances have no effect on genotyping.

Interferent	Concentration	# samples tested	First Run Results				Final Results[a]			
			# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)	# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)
Control	N/A	15	15	0	0	100	15	0	0	100
Amylase	311-341 U	15	15	0	0	100	15	0	0	100
Hemoglobin	21.2-22.1 mg/mL	15	14	1	0	93.3	15	0	0	100
IgA	300-500 mg/L	15	15	0	0	100	15	0	0	100
Total Protein	2.1-2.7 mg/mL	15	13	2	0	86.7	15	0	0	100

[a]Final Results reflect a second repeat run of samples with no calls during the first run
[b]Correct call rate = # correct calls / # of samples tested

Exogenous Interferents

Potentially interfering exogenous substances (eating, drinking, chewing gum, mouthwash and smoking) introduced into saliva samples collected using the Oragene® Dx collection device OGD-500 through various activities were tested. Each activity group was composed of five donors who each provided two samples: a baseline/control sample prior to the activity, and a sample collected 30 minutes after the activity. DNA was extracted using the Qiagen QIAamp DSP DNA Mini Kit. Samples were tested in triplicate. Results from the TruDiagnosis® System were compared to the genotype for each sample as determined by bidirectional Sanger sequencing.

Results from the testing are summarized below. There was 100% agreement between the TruDiagnosis® System and bidirectional DNA sequencing for all activities following a first run, or a second run for no calls observed during the first run, supporting that these activities have no effect on genotyping after 30 minutes.

Activity	Timepoint	# samples tested	First Run Results				Final Results[a]			
			# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)	# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)
Eating	Baseline	15	14	1	0	93.3	15	0	0	100
	30 minutes	15	15	0	0	100	15	0	0	100
Drinking	Baseline	15	15	0	0	100	15	0	0	100
	30 minutes	15	15	0	0	100	15	0	0	100
Chewing Gum	Baseline	15	15	0	0	100	15	0	0	100
	30 minutes	15	13	2	0	86.7	15	0	0	100
Mouthwash	Baseline	15	15	0	0	100	15	0	0	100
	30 minutes	15	15	0	0	100	15	0	0	100
Smoking	Baseline	15	6	9	0	40	15	0	0	100
	30 minutes	15	9	6	0	60	15	0	0	100

[a]Final Results reflect a second repeat run of samples with no calls during the first run
[b]Correct call rate = # correct calls / # of samples tested

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison:

The method comparison evaluation was performed to assess the genotyping accuracy of the TruDiagnosis® System as compared to bi-directional DNA sequencing. 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. DNA was extracted using the Qiagen QIAamp DSP DNA Mini Kit.

The study was performed by four operators using four lots of the TruArray® Warfarin Sensitivity Test Kit and four TruDx® 2000 Imagers over 14 testing 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.

Agreement between the TruDiagnosis® System and bi-directional DNA sequencing (by loci):

Genotype[a]	# samples tested	# correct calls[b]	# no calls	# in-correct calls	Agreement	95% One-Sided Confidence Lower Limit
2C9*2 *1/*1	227	227	0	0	100	98.7
95.6274.C9*2 *1/*2	66	66	0	0	100	95.6
2C9*2 *2/*2	10	10	0	0	100	74.1
2C9*2 Total	303	303	0	0	100	98.8
2C9*3 *1/*1	247	247	0	0	100	98.8
2C9*3 *1/*3	55	54	1	0	98.2	91.7
2C9*3 *3/*3	1	1	0	0	100	5.0
2C9*3 Total	303	302	1	0	99.6	98.2
VKORC1 -1639 AA	102	101	1	0	99	95.4
VKORC1 -1639 GA	126	125	0	1	99.2	96.3
VKORC1 -1639 GG	75	74	1	0	98.7	93.8
VKORC1 -1639 Total	303	301	1	1	99.3	97.6

[a]Genotype determined by bidirectional DNA sequencing
[b]Calls produced on first run

Agreement between the TruDiagnosis® System and bi-directional DNA sequencing (by sample):

Genotype			# samples tested	First Run Results				Final Results[a]			
CYP 2C9*2	CYP 2C9*3	VKORC1 -1639		# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)	# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)
*1/*1	*1/*1	AA	58	57	1	0	98.3	58	0	0	100
*1/*1	*1/*1	GA	79	79	0	0	100	79	0	0	100
*1/*1	*1/*1	GG	41	41	0	0	100	41	0	0	100
*1/*2	*1/*1	AA	8	8	0	0	100	8	0	0	100
*1/*2	*1/*1	GA	21	21	0	0	100	21	0	0	100
*1/*2	*1/*1	GG	30	29	1	0	96.7	30	0	0	100
*1/*1	*1/*3	AA	8	7	1	0	87.5	8	0	0	100
*1/*1	*1/*3	GA	15	15	0	0	100	15	0	0	100

Genotype			# samples tested	First Run Results				Final Results[a]			
CYP 2C9*2	CYP 2C9*3	VKORC1 -1639		# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)	# correct calls[b]	# no calls	# in-correct calls	Correct call rate (%)
*1/*1	*1/*3	GG	25	25	0	0	100	25	0	0	100
*2/*2	*1/*1	AA	1	1	0	0	100	1	0	0	100
*2/*2	*1/*1	GA	6	5	0	1	83.3	6	0	1	83.3
*2/*2	*1/*1	GG	3	3	0	0	100	3	0	0	100
*1/*1	*3/*3	GG	1	1	0	0	100	1	0	0	100
*1/*2	*1/*3	GA	6	6	0	0	100	6	0	0	100
*1/*2	*1/*3	GG	1	1	0	0	100	1	0	0	100
Total			303	299	3	1	98.7	303	0	1	99.6

[a]Final Results reflect a second repeat run of samples with no calls during the first run
[b]Correct call rate = # correct calls / # of samples tested

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

In the package insert, the sponsor includes the following allele frequencies across ethnic groups for each allele which have been shown to affect an individual's sensitivity to warfarin.

Allele	Ethnicity		
	Caucasian	African	Asian
CYP2C9*2	0.9-20%	0.8-7%	0%
CYP2C9*3	0-14.5%	0.4-3%	0-8.2%
VKORC1 -1639	37%	14%	89%

N. Instrument Name:

TruDx® 2000 Imager

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No __X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   The TruDx® 2000 Imager comes equipped with a barcode scanner and stand to scan sample accession IDs and the barcodes identified on the Test Slide. Alternatively, the sample identification can be entered manually.

4. Specimen Sampling and Handling:

   Human genomic DNA from saliva collected using the Oragene® Dx OGD 500 Device and extracted using Qiagen QIAamp DSP DNA Mini Kit.

5. Calibration:

   The instrument does not require calibration. Optical system checks should be performed periodically to ensure the accuracy of the imager. Optical System checks are performed

using the Uniformity Test Slide included with the TruDx® 2000 Imager. The user can select Run Optical System Check from the Configuration screen to initiate the analysis workflow for the optical system check.

6. Quality Control:

   An external positive and negative control should be processed with each run of Test Slides to ensure the integrity of sample preparation, kit reagents, and individual laboratory practices. The external positive control(s) should be 25 ng human gDNA of known *2, *3 and VKORC1 genotype; the external negative control should be a reagent blank (e.g., nuclease-free water).

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable and the special controls for this device type under 21 CFR 862.3360 and 21 CFR 862.2570.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.