EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
ARK Voriconazole II Assay Test System

DECISION SUMMARY

A. DEN Number:

DEN160033

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation of the ARK Voriconazole II Assay Test System

C. Measurand:

Voriconazole

D. Type of Test:

Homogenous Enzyme Immunoassay

E. Applicant:

ARK Diagnostics, Inc.

F. Proprietary and Established Names:

ARK Voriconazole II Assay Test System includes the following:
ARK Voriconazole II Assay
ARK Voriconazole II Calibrator
ARK Voriconazole II Control

G. Regulatory Information:

1. Regulation:

   21 CFR 862.3970

2. Classification:

   Class II (Special Controls)

3. Product code:

   PUJ

4. Panel:

   91 – Toxicology

H. Indications for use:

1. Indication(s) for use:

   ARK Voriconazole II Assay Test System includes separately provided test kits for the ARK Voriconazole II Assay, ARK Voriconazole II Calibrator, and ARK Voriconazole II Control.

   The ARK Voriconazole II Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of voriconazole in human serum on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of voriconazole to help ensure appropriate therapy. The assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

   ARK Voriconazole II Calibrator is intended for use in calibration of the ARK Voriconazole II Assay.

   ARK Voriconazole II Control is an assayed quality control material intended for use in quality control of the ARK Voriconazole II Assay.

2. Special conditions for use statement(s):

   For prescription use only.

   The assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

3. Special instrument requirements:

   The assay was validated on the Roche cobas c 501 analyzer

I. Device Description:

The ARK Voriconazole II Assay Test System consists of the ARK Voriconazole II Assay, the ARK Voriconazole II Calibrator, and the ARK Voriconazole II Control.

The ARK Voriconazole II Assay consists of:

- Reagent R1: rabbit polyclonal antibodies to voriconazole, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers.
- Reagent R2: voriconazole labeled with bacterial G6PDH buffer, bovine serum albumin, sodium azide, and stabilizers.

The ARK Voriconazole II Calibrator has six levels and consists of voriconazole, buffer, bovine serum albumin, and sodium azide.

The ARK Voriconazole II Control has three levels and consists of voriconazole, buffer, bovine serum albumin, and sodium azide.

J. Standards/Guidance Documents Referenced:

- CLSI document EP5-A3, Evaluation of Precision of Quantitative Measurement Procedures
- CLSI Guideline EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
- CLSI Protocol EP7-A2: Interference Testing in Clinical Chemistry
- CLSI Guideline EP9-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI Protocol EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

K. Test Principle:

ARK Voriconazole II Assay is a homogeneous enzyme immunoassay based on competition between drug in the specimen and voriconazole-conjugated glucose-6-phosphate dehydrogenase (voriconazole-G6PDH) for binding to the antibody reagent. As voriconazole-G6PDH binds antibody, voriconazole-G6PDH enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active voriconazole-G6PDH enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous plasma G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

L. Performance Characteristics:

The following performance characteristics were obtained on the Roche cobas c 501 analyzer.

1. <u>Analytical performance:</u>

a. Reproducibility/Precision

Internal Precision Study:

An internal 20-day precision study was performed by testing tri-level controls and three samples from pooled human serum. Each level was tested over 20 days, with 2 runs per day and 4 replicates per run. The following results were obtained:

Sample	N	Mean (µg/mL)	Within Run		Between Run		Between Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Voriconazole II Control										
LOW	160	1.03	0.047	4.6	0.030	2.9	0.022	2.1	0.051	4.9
MID	160	4.91	0.194	3.9	0.124	2.5	0.101	2.1	0.209	4.3
HIGH	160	9.39	0.394	4.2	0.242	2.6	0.207	2.2	0.426	4.5
Human Serum										
LOW	160	1.02	0.043	4.2	0.029	2.8	0.024	2.4	0.047	4.6
MID	160	5.03	0.182	3.6	0.149	3.0	0.111	2.2	0.217	4.3
HIGH	160	9.80	0.334	3.4	0.286	2.9	0.221	2.3	0.407	4.2

Another internal precision study was performed by testing three voriconazole patient sample pools using three lots of the ARK Voriconazole II Assay reagents. Each level was tested over 5 days, with 2 runs per day and 4 replicates per run. The following results were obtained:

Sample	N	Mean (µg/mL)	Within Run		Between Run		Between Day		Between Lot		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LOW	120	1.00	0.042	4.2	0.023	2.3	0.021	2.1	0.034	3.4	0.059	5.9
MID	120	4.83	0.234	4.8	0.069	1.4	0.048	1.0	0.102	2.1	0.248	5.1
HIGH	120	10.59	0.621	5.9	0.293	2.8	0.266	2.5	0.096	0.9	0.667	6.3

Multi-Site Precision Study

Precision was also evaluated at three sites (ARK and two external sites) over 5 non-consecutive days. ARK tri-level voriconazole controls and three samples from pooled human serum were tested over 5 days, with 2 runs per day and 4 replicates per run. The overall (across sites) precision study results are shown below:

Sample	N	Mean (µg/mL)	Repeatability		Between-Run		Between-Day		Reproducibility	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Voriconazole II Control										
LOW	120	1.08	0.054	5.0	0.044	4.1	0.036	3.4	0.064	6.0
MID	120	5.04	0.247	4.9	0.171	3.4	0.144	2.9	0.275	5.5
HIGH	120	9.93	0.466	4.7	0.480	4.8	0.320	3.2	0.645	6.5
Human Serum										
LOW	120	1.06	0.047	4.4	0.040	3.8	0.032	3.0	0.057	5.4
MID	120	5.12	0.251	4.9	0.148	2.9	0.120	2.3	0.265	5.2
HIGH	120	10.13	0.510	5.0	0.338	3.3	0.292	2.9	0.561	5.5

b. Linearity/assay reportable range:

Linearity

Pure voriconazole (USP, 99.7% purity) was added to pooled human serum to obtain a concentration of 20.0 µg/mL. Dilutions of this 20.0 µg/mL high concentration sample were made proportionally using human serum negative for voriconazole. Eleven levels of samples were tested in two runs with three replicates per run. Regression analysis was performed between the measured mean voriconazole and calculated values for each dilution. The linear regression results are shown below.

$Y = 1.0209 X – 0.0416$
R^2: 0.9995

These results support the claimed measuring range of 0.5 to 14.0 µg/mL.

Analytical Recovery

Analytical recovery throughout the measurement range was assessed. Serum samples were prepared by gravimetric addition of pure voriconazole (USP, 99.7% purity) to methanol and volumetric addition of this stock solution to human serum negative for voriconazole. The percentage recovery ranged from 90.0% to 104.9%, as shown below:

Calculated Concentration (µg/mL)	Measured Concentration (µg/mL)	Percent Recovery
0.50	0.45	90.0
1.20	1.19	99.2
3.00	3.05	101.7
6.00	5.86	97.7
9.00	8.74	97.1
12.00	11.44	95.3
15.00	15.75	104.9

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

Traceability
The ARK Voriconazole II Calibrators are traceable to a certified USP Reference Standard.

Value Assignment
Concentrations of the ARK Voriconazole II Calibrators and Controls are assigned through internal procedures that were reviewed and found to be acceptable.

Stability:
Accelerated stability studies were performed to support a shelf-life stability claim of up to 12 months for the ARK Voriconazole II Reagents, Calibrators and Controls when stored unopened at 2-8°C. Real time stability studies to support shelf-life stability claims are ongoing. Real-time testing for on-board stability of ARK Voriconazole II Reagents was performed and supports an in-use stability claim of 60 days for reagents. Specimen stability was also evaluated and was shown to be stable for up to a week at 2-8°C and up to four weeks at -20 °C.

d. Detection limit

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with CLSI EP17-A2.

The Limit of Blank (LoB) was evaluated by testing 20 blank patient specimens over 3 days using 3 lots of ARK Voriconazole II reagents, and was determined to be 0.003 ug/mL.

The Limit of Detection (LoD) was calculated based on the LoB and the standard deviation of a low concentration pooled serum sample tested over 3 days using 3 lots of ARK Voriconazole II reagents, and was determined to be 0.05 ug/mL.

The Limit of Quantitation (LoQ) was defined as the lowest Voriconazole concentration that shows bias ≤15% and within-laboratory precision of ≤ 10%, and was determined to be 0.5 ng/mL.

e. Analytical specificity:

Interference studies were conducted using CLSI Protocol EP7-A2 as a guideline. Clinically high concentrations of potentially interfering substances were spiked into serum samples with known levels of voriconazole (approximately 1 and 5 μg/mL). Each sample was then tested in two runs with three replicates per run using the ARK Voriconazole II Assay, along with a serum control of voriconazole. The percentage recovery relative to the measurement of voriconazole in the serum control was calculated. The sponsor states that interference is considered to be significant if the

analytical recovery is outside of ± 10% of the initial value.

Endogenous Substances

Interference studies were conducted for endogenous substances with serum samples at 1.0 and 5.0 µg/mL of voriconazole. The concentration tested for each endogenous substance and percent recovery results are shown below.

Interfering Substance	Interferent concentration tested	Percentage Recovery	
		1.0 µg/mL Voriconazole	5.0 µg/mL Voriconazole
Human Albumin	12 g/dL	103.8	98.7
Bilirubin (Conjugated)	70 mg/dL	99.0	99.6
Bilirubin (Unconjugated)	70 mg/dL	102.9	95.7
Cholesterol	617 mg/dL	95.8	98.3
Hemoglobin	1000 mg/dL	102.9	93.5
Rheumatoid Factor	1000 IU/mL	103.8	100.2
Human IgG	12 g/dL	105.8	97.8
Triglycerides	1000 mg/dL	107.5	99.2
Uric Acid	30 mg/dL	105.8	96.2

Cross-reactivity of N-oxide Voriconazole

A cross-reactivity study was conducted for the major metabolite of voriconazole, N-oxide-voriconazole at 5.0 µg/mL and 10.0 µg/mL levels. The results demonstrated non-significant (≤ 3.0%) cross reactivity from N-oxide-voriconazole when tested in the absence or presence of voriconazole in human serum.

N-oxide-Voriconazole (µg/mL)	Measured Voriconazole in Absence/Presence of Metabolite (µg/mL)		
	Voriconazole Absent (0.0 µg/mL)	Voriconazole Present (1.0 µg/mL)	Voriconazole Present (5.0 µg/mL)
0.0	0.00	1.06	4.99
5.0	0.04	1.17	4.96
10.0	0.10	1.23	5.29

Potentially Co-Administered Medications

Interference studies were conducted for structurally related compounds, medications routinely co-administered with voriconazole, and other potentially co-administered drugs, at serum concentrations of 1.0 and 5.0 µg/mL of voriconazole. The concentration tested for each potentially interfering compound and percent recovery results are shown below. No significant interference was observed.

#	Compound	Concentration tested (μg/mL)	Percentage Recovery	
			1.0 μg/mL Voriconazole	5.0 μg/mL Voriconazole
1	Abacavir	30	100	96.6
2	Acetaminophen	200	100	90.9
3	Alprazolam	5	100	97.4
4	Amikacin	100	99	100.4
5	Amphotericin	100	92.5	96.7
6	Amprenavir	30	99	96.4
7	Atazanavir	30	102.1	95.4
8	Atovaquone	100	94.8	93
9	Bendamustine	30	98	96.4
10	Bosutinib	100	104.1	94.6
11	Cefepime	500	96.9	92.8
12	Ceftazidime	500	99	93.8
13	Ciprofloxacin	100	96.1	96
14	Citalopram	10	100	95.4
15	Clonazepam	10	99	99.2
16	Codeine	10	99	102
17	Colistimethate Na	100	99	98.4
18	Cyclosporine A	40	99	99.2
19	Darunavir	30	100	94.3
20	Dasatinib	100	99	100.8
21	Efavirenz	30	95.1	93.9
22	Emtricitabine	30	99	92.6
23	Erythromycin	200	99	91.5
24	Fluconazole	30	101.8	97.2
25	Fosamprenavir	30	97	100.4
26	Gabapentin	100	98.2	95.3
27	Gentamicin	100	98.1	100
28	Itraconazole	20	97.2	93.4
29	Lamivudine	30	96.3	99.2
30	Lopinavir	30	94.4	99.2
31	Lorazapam	10	98.1	101
32	Maraviroc	10	99.1	100.8
33	Meropenem	500	92.5	96.4
34	Methotrexate	100	95.3	94.4
35	Metronidazole	200	93.4	95.8
36	Micafungin	300	98.1	101.2
37	Morphine	10	96.3	91.7
38	Mycophenolic acid	40	97	91.1
39	Nelfinavir	30	93.4	94.7
40	Nevirapine	30	94.4	94.3

#	Compound	Concentration tested (μg/mL)	Percentage Recovery	
			1.0 μg/mL Voriconazole	5.0 μg/mL Voriconazole
41	Olanzapine	10	98	91.1
42	Penicillin V	100	98.1	102.4
43	Piperacillin	500	93.1	92.2
44	Posaconazole	20	99.1	93
45	Prednisolone	200	102.9	91.3
46	Ritonavir	30	100	99.2
47	Sirolimus	10	104.7	101.6
48	Stavudine	30	96.3	101.5
49	Tazobactam	100	99	90.2
50	Tacrolimus	10	98	94.6
51	Tenofovir	30	98	96.7
52	Tipranavir	30	96.3	102.4
53	Tobramycin	100	100	96.3
54	Trimethoprim	50	93.9	91.8
55	Sulfamethoxazole	400	97	91.8
56	Vancomycin	250	98.2	101.2
57	Vincristine	100	93.1	92
58	Zolpidem	30	102.8	91.2

2. Comparison studies:

a. Method comparison study:

Method comparison studies were performed following CLSI Protocol EP9-A3 at 3 study sites. Results from 165 serum specimens tested with the ARK Voriconazole II Assay on Roche cobas c 501 analyzers at 3 sites were compared to those from a validated LC-MS/MS method. The samples represented a diverse population of in-hospital patients with voriconazole concentrations ranging from 0.5 to 13.9 μg/mL by the LC-MS/MS method. The Passing Bablok regression analysis results are presented below.

Site	N	Slope (95% CI)	Intercept (95% CI)	R^2 (95% CI)	Sample Range Tested (concentration on LC-MS/MS)
1	165	0.99 (0.96 to 1.03)	0.08 (0.04 to 0.16)	0.96 (0.94 to 0.96)	0.5 to 13.9 μg/mL
2		0.99 (0.96 to 1.02)	0.05 (-0.03 to 0.15)	0.95 (0.93 to 0.96)	
3		0.98 (0.95 to 1.01)	0.06 (-0.01 to 0.11)	0.97 (0.95 to 0.97)	

b. Matrix comparison:

Not applicable

3. Clinical studies:

Not applicable

4. Expected Values

Not applicable.

M. Labeling

The labeling is sufficient and satisfies the requirements of 21 CFR Parts 801 and 809, and the special controls for this type of device.

N. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	**Identified Mitigations**
Clinical action (e.g., dose adjustments) based on falsely elevated inaccurate voriconazole results may lead to decreased clinical efficacy of the drug and consequently poorer clinical outcomes.	General controls and special controls (1), and (2)
Clinical action (e.g. dose adjustments) based on falsely low inaccurate voriconazole results may lead to an increased risk of toxicity.	General controls and special controls (1), and (2)

O. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	Voriconazole is characterized by a narrow therapeutic spectrum, non-linear pharmacokinetics, and frequent inter-individual and intra-individual variability in voriconazole serum concentrations. It is metabolized in the liver by several cytochrome P450 isoenzymes; however, voriconazole is primarily metabolized by CYP2C19. The CYP2C19 gene is known to have several different genetic polymorphisms which are known to differentially affect voriconazole metabolism. For these above-mentioned reasons, voriconazole therapeutic drug monitoring (TDM) can assist clinicians in determining adequate dose exposure levels when evaluating patients' overall clinical response. Clinical decision making should not be based solely on review of voriconazole TDM when assessing patients' risk of drug toxicity or therapeutic benefit. Voriconazole TDM should be used only as an adjunct to clinical care and not as a sole means of determining clinical response.

Summary of the Risk(s)	Although, there is generally no accepted voriconazole TDM target range, several studies propose voriconazole TDM cut-off ranges anywhere from 1-5.5 µg/mL or 2-6 µg/mL, where a trough of 2 µg/mL is generally acceptable among patients with more severe illness. Studies have linked voriconazole levels of <1.0 µg/mL with therapeutic failure (Chen J, et al., Therapeutic drug monitoring of voriconazole in children, Ther Drug Monit 2012; 34:77-84). Therefore, the risk of a falsely elevated voriconazole value is decreased clinical efficacy of the drug and consequently poorer clinical outcomes, as the physician may erroneously believe that voriconazole is within an effective range based on the inaccurate voriconazole TDM value. This is of particular concern for individuals with life-threatening fungal infections, among whom the proportion of patients with potentially sub-therapeutic levels may be high (Trifilio S, et al., Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients, Cancer 2007; 109:1532-1535). A falsely elevated TDM value could result in a physician prematurely discontinuing voriconazole therapy out of concern that at elevated levels the patient may be subject to increased toxicity (Potoski B, Brown J, The safety of voriconazole, Clin Infect Dis 2002; 35: 1273-1275; Lutsar I, et al., Voriconazole treatment for less-common, emerging, or refractory fungal infections, Clin Infect Dis 2003; 36: 1122-1131). The risk of a falsely low voriconazole value is increased risk of toxicity if voriconazole is continued at the same dose, or a potential increase in the dose based on a falsely low level. The population of patients taking voriconazole generally has significant co-morbidities (e.g., hematopoietic stem cell transplant patients at increased risk for graft-versus-host disease (GVHD) or patients may be receiving concomitant medications which are also metabolized through the same metabolic pathways as voriconazole). Therefore, physicians may opt to routinely follow clinically relevant biochemical parameters, such as liver enzyme studies, to ensure that there is no worsening of these parameters. Therapeutic dose levels should be monitored alongside patients' overall clinical progress, as false readings in any direction may adversely affect patients' clinical outcomes. Voriconazole levels should be used as an adjunct to clinical judgment and clinical monitoring and not as a sole means of assessing the adequacy of voriconazole dosing, or voriconazole toxicity, or as a surrogate for patient outcomes.
Summary of Other Factors	As specified in the label, a voriconazole level may be used as an adjunct to clinical judgment and clinical monitoring and should not be used as a sole means of assessing the adequacy of voriconazole dosing, or toxicity. Additionally, analytical risks are mitigated by in vitro diagnostic (IVD) labelling compliant with 21 CFR 809.10.

Conclusions Do the probable benefits outweigh the probable risks?
Yes, the probable benefits outweigh the probable risks of this device in light of the special controls assigned, along with general controls, including design controls.

Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

P. Conclusion:

Product Code: PUJ
Device Type: Voriconazole test system
Class: II (special controls)
Regulation: 21 CFR 862.3970

a) *Identification.* A voriconazole test system is a device intended to measure voriconazole in human serum. Measurements obtained by this device are used in monitoring levels of voriconazole to ensure appropriate therapy.

b) *Classification*. Class II (special controls). A voriconazole test system must comply with the following special controls:

1) Premarket notification submissions must include the following information:
   A. Data demonstrating the precision of the voriconazole test system. Precision studies must include a minimum of three samples containing different concentrations of voriconazole, including near medical decision points at the high and low end of the expected therapeutic range. Samples with concentrations near medical decision points must be clinical specimens collected from patients taking voriconazole.
   B. Method comparison data demonstrating accuracy of the voriconazole test system. Method comparison data must be collected at three laboratory sites. The comparator method must not be subject to bias due to non-specific detection of voriconazole.
   C. Data from interference studies performed to evaluate potential interference from co-administered medications used for conditions in which voriconazole is indicated.
   D. Data from studies performed to evaluate cross reactivity of the major metabolite, *N*-oxide voriconazole.

2) Your 809.10(b)(5)(ii) compliant labeling must include a warning statement as follows: "This assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures."