

For Export Only – Not For Sale in USA

ARK™ Voriconazole II Assay

This ARK Diagnostics, Inc. package insert for the ARK Voriconazole II Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE











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KEY TO SYMBOLS USED

	Batch code	 YYYY-MM-DD	Use by/Expiration date
	Catalog Number		Manufacturer
	Authorized Representative		CE Mark
	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use		Reagent 1/ Reagent 2

1 NAME

ARK™ Voriconazole II Assay

2 INTENDED USE

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

3 SUMMARY AND EXPLANATION OF THE TEST

Voriconazole (VFEND®, Pfizer) is a triazole antifungal agent and is designated chemically as (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol.¹ VFEND is a triazole antifungal drug indicated for use in the treatment of:

- Invasive aspergillosis
- Candidemia (nonneutropenics) and disseminated candidiasis in skin, abdomen, kidney, bladder wall, and wounds
- Esophageal candidiasis
- Serious infections caused by *Scedosporium apiospermum* and *Fusarium* spp. including *Fusarium solani*, in patients intolerant of, or refractory to, other therapy

4 PRINCIPLES OF THE PROCEDURE

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

5 REAGENTS

REF	Product Description	Quantity/Volume
5030-0001-01	ARK Voriconazole II Assay Reagent R1 – Antibody/Substrate rabbit polyclonal antibodies to voriconazole, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Voriconazole labeled with bacterial G6PDH, buffer, bovine serum albumin, sodium azide, and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Voriconazole II Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.** ARK Voriconazole products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use. For prescription use only.
- Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Reagents contain ≤0.09% sodium azide.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum is required. For consistency, using the same specimen matrix for individual patients is a good practice. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of voriconazole. Time of blood draw since last dose should be noted.
- Whole blood cannot be used. The following anticoagulants were shown not to interfere with this assay.
 - Sodium heparin
 - Lithium heparin
 - Potassium EDTA

- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- **Handle all patient specimens as if they were potentially infectious.**

8 PROCEDURE

Materials Provided

ARK Voriconazole II Assay – [REF] 5030-0001-01

Materials Required – Provided Separately

ARK Voriconazole II Calibrator – [REF] 5030-0002-01

Quality Controls – ARK Voriconazole II Control – [REF] 5030-0003-01

Instruments

Reagents [R1] and [R2] may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of [R1] and [R2].

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual and instrument-specific application sheet.

Calibration

Perform a calibration using the ARK Voriconazole II Calibrators A, B, C, D, E, and F. Calibration is required with each new reagent kit lot number. Verify the calibration curve with quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC)

Follow QC procedures for the ARK Voriconazole II Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Manual Dilution Protocol

The measurement range of the ARK Voriconazole II Assay is 0.5 – 16.0 µg/mL. Specimens containing voriconazole in higher concentrations (>16.0 µg/mL) are assayed by dilution of the specimen into the measurement range. Dilute the specimen with zero calibrator (CAL A). A four-fold dilution factor is suggested. Multiply the assayed result by the dilution factor.

$$\text{Manual Dilution Factor} = \frac{\text{Volume of Specimen} + \text{Volume of CAL A}}{\text{Volume of Specimen}}$$

9 RESULTS

Report result units as µg/mL or µmol/L. To convert results from µg/mL voriconazole to µmol/L voriconazole, multiply µg/mL by 2.863. The voriconazole value from this assay should be used in conjunction with other clinical information. Refer to the instrument specific operator's manual for any result error codes.

10 LIMITATIONS OF PROCEDURE

This assay is designed for use with serum; refer to the sections **Specimen Collection and Preparation for Analysis**. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variabilities. See the section **Expected Values** below.

11 EXPECTED VALUES

A therapeutic range for voriconazole has not been well established. The reference range of 1.0 µg/mL to 5.5 µg/mL has been proposed.² Steady state concentrations may be achieved after 5 to 7 days of treatment. Practice guidelines³⁻⁵ support TDM and clinical application of TDM has been recommended⁶⁻⁸ due to the high inter-individual and intra-individual variation in the metabolism⁹⁻¹⁰ of voriconazole, non-linear pharmacokinetics and CYP2C19 polymorphisms.¹¹⁻¹² Voriconazole treatment has been used for invasive fungal infection and prophylactically in transplant patients.¹³⁻¹⁵ Consideration should be given to the requirements for pediatric use, since metabolism in children may be different than for adults.¹⁶⁻¹⁸

Voriconazole drug concentrations should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy and dosage adjustments.

12 SPECIFIC PERFORMANCE CHARACTERISTICS

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche cobas c 501 System.

Sensitivity

Limit of Quantitation (LOQ)

The following characteristics were determined according to CLSI EP17-A2 for the ARK Voriconazole II Assay. Analyzer-specific performance may vary.

Criterion	Voriconazole Concentration (µg/mL)
Limit of Blank (LoB); N = 60 µB + 1.645 SD, where SD = 0.0000	0.005
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.0116	0.024
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD With acceptable recovery and linearity	0.50

Each laboratory is responsible for determining reporting criteria for voriconazole concentrations. The following suggestion from CLSI EP17-A2 may be appropriate:

Result ≤ LoB report "not detected; concentration < LoD"

LoB < Result < LoQ report "analyte detected; concentration < LoQ"

Result ≥ LoQ report the result as measured

Measurement Range

The measurement range of the ARK Voriconazole II Assay is 0.5 – 16.0 µg/mL. Specimens containing voriconazole in higher concentrations (>16.0 µg/mL) are assayed by dilution of the specimen into the measurement range. Refer to **Section 8 Procedure - Manual Dilution Protocol**.

Recovery

Analytical recovery was assessed by adding concentrated voriconazole drug into human serum negative for voriconazole. A stock concentrate of voriconazole in dimethylformamide (DMF) was added volumetrically to human serum negative for voriconazole, representing drug concentrations across the assay range. Six replicates of each sample were assayed. The results were averaged and compared to the target concentration and percent recovery calculated.

$$\% \text{ Recovery} = \frac{100 \times \text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
0.5	0.45	90.0
1.2	1.19	99.2
3.0	3.05	101.7
6.0	5.86	97.7
9.0	8.74	97.1
12.0	11.44	95.3
15.0	15.75	105.0

Mean percent recovery: 98.0

Linearity

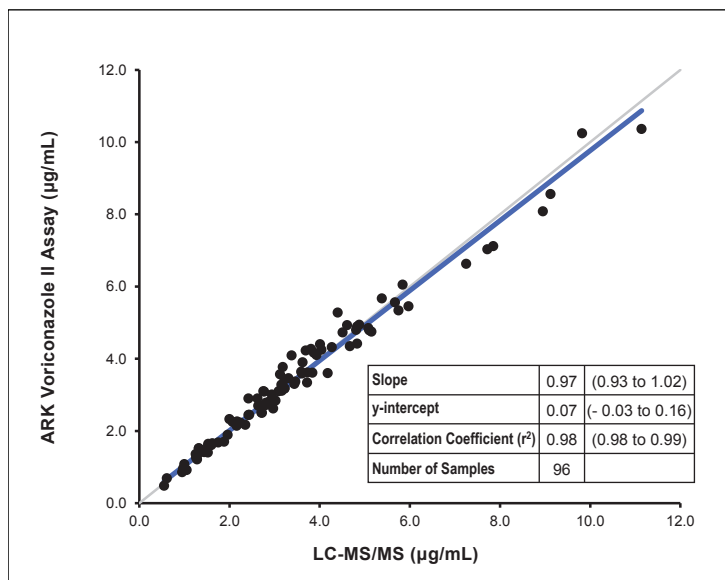
Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 20.0 µg/mL voriconazole serum sample was prepared and dilutions were made proportionally with human serum negative for voriconazole. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regression values, or ≤ 0.2 µg/mL at concentrations ≤ 2.0 µg/mL. A linear relationship was demonstrated between 0.5 and 16.0 µg/mL.

Nominal (µg/mL)	Measured Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Difference (%)
0.0	0.00	-0.04	0.02	NA
0.5	0.43	0.47	0.51	0.04 µg/mL
1.0	1.02	0.98	1.01	0.03 µg/mL
2.0	2.05	2.00	2.00	0.00 µg/mL
4.0	4.21	4.04	4.00	-1.0%
6.0	5.89	6.08	6.02	-1.1%
8.0	8.08	8.13	8.06	-0.9%
10.0	9.91	10.17	10.11	-0.6%
12.0	12.26	12.21	12.18	-0.2%
14.0	14.43	14.25	14.28	0.2%
16.0	16.31	16.29	16.39	0.6%
20.0*	21.94	NA	NA	NA

*Above the measurement range

Method Comparison

Method comparison studies were performed using CLSI Protocol EP9-A3 as a guideline. Results from the ARK Voriconazole II Assay were compared with results from LC-MS/MS for sera obtained from 96 patients with voriconazole concentrations ranging from 0.6 µg/mL to 11.1 µg/mL. Passing-Bablok¹⁹ regression statistics are shown below (with 95% confidence limits).



Precision

Precision was determined as described in CLSI Protocol EP5-A3. Tri-level controls and three human serum pooled specimens containing voriconazole were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV.

Sample	N	Mean (µg/mL)	Within Run		Between Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Voriconazole Control								
LOW	160	1.04	0.048	4.7	0.028	2.7	0.055	5.3
MID	160	5.09	0.196	3.8	0.158	3.1	0.255	5.0
HIGH	160	9.83	0.541	5.5	0.303	3.1	0.641	6.5
Human Serum								
LOW	160	1.02	0.045	4.4	0.035	3.4	0.062	6.0
MID	160	5.05	0.225	4.5	0.175	3.5	0.293	5.8
HIGH	160	9.91	0.493	5.0	0.395	4.0	0.633	6.4

Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of voriconazole (1.0 and 5.0 µg/mL) were evaluated. Each sample was assayed using the ARK Voriconazole II Assay, along with a serum control of voriconazole. Measurement of voriconazole resulted in ≤10% error in the presence of interfering substances at the levels tested.

Interfering Substance	Interferent Concentration	Percentage Recovery	
		1.0 µg/mL Voriconazole	5.0 µg/mL Voriconazole
Albumin	12 g/dL	104.5	98.8
Bilirubin - conjugated	70 mg/dL	99.4	99.7
Bilirubin - unconjugated	70 mg/dL	103.4	95.9
Cholesterol	300 mg/dL	101.9	92.6
Gamma-Globulin	12 g/dL	106.3	97.9
Hemoglobin	1000 mg/dL	103.0	93.5
Rheumatoid Factor	1000 IU/mL	103.5	100.1
Triglycerides	1000 mg/dL	107.2	99.2
Uric Acid	30 mg/dL	105.5	96.2

Specificity

Metabolism

Voriconazole displays highly variable non-linear pharmacokinetics, which are primarily due to polymorphic CYP2C19 metabolism. After oral and intravenous administration voriconazole is extensively metabolized to inactive metabolites including N-oxide voriconazole, 4-hydroxy-voriconazole and di-hydroxy-voriconazole. Voriconazole and its major metabolite, N-oxide voriconazole, are present quantitatively in serum²⁰, while hydroxylated metabolites are easily excreted into urine. The concentration of N-oxide metabolite usually does not exceed concentration of the parent drug. Pharmacokinetics of voriconazole may be further influenced by other drug metabolizing enzymes and age-based differences in drug metabolism.

Metabolite

The crossreactivity of N-oxide voriconazole metabolite (5.0 µg/mL or 10.0 µg/mL) in the ARK Voriconazole II Assay was not clinically significant (≤ 3.0% crossreactivity) when tested in the absence (0.0 µg/mL) or presence of voriconazole (1.0 µg/mL or 5.0 µg/mL) in human serum.

N-oxide-VRZ (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

Crossreactivity

The compounds listed below did not interfere with the ARK Voriconazole II Assay when tested in the absence (0.0 µg/mL) or presence of voriconazole (1.0 µg/mL and 5.0 µg/mL). Levels tested were at or above maximum physiological or pharmacological concentrations. Voriconazole concentrations of samples containing interferent were compared to the voriconazole level in a normal serum control.

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Abacavir	30	Lopinavir	30
Acetaminophen	200	Lorazepam	10
Alprazolam	5	Maraviroc	10
Amikacin	100	Meropenem	500
Amphotericin	100	Methotrexate	100
Amprenavir	30	Metronidazole	200
Atazanavir	30	Micafungin	300
Atovaquone	100	Morphine	10
Bendamustine	30	Mycophenolic acid	40
Bosutinib	100	Nelfinavir	30
Cefepime	500	Nevirapine	30
Ceftazidime	500	Olanzapine	10

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Ciprofloxacin	100	Penicillin V	100
Citalopram	10	Piperacillin	500
Clonazepam	10	Posaconazole	20
Codeine	10	Prednisolone	200
Colistimethate sodium	100	Ritonavir	30
Cyclosporin A	40	Sirolimus	10
Darunavir	30	Stavudine	30
Dasatinib	100	Tazobactam	100
Efavirenz	30	Tacrolimus	10
Emtricitabine	30	Tenofovir	30
Erythromycin	200	Tipranavir	30
Fluconazole	30	Tobramycin	100
Fosamprenavir	30	Trimethoprim	50
Gabapentin	100	Sulfamethoxazole	400
Gentamicin	100	Vancomycin	250
Itraconazole	20	Vincristine	100
Lamivudine	30	Zolpidem	30

13 REFERENCES

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14 TRADEMARKS

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