ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ *Linezolid Assay*

This ARK Diagnostics, Inc. package insert for the ARK Linezolid 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. The ARK Linezolid Assay test system includes separately provided test kits for the ARK Linezolid Assay, ARK Linezolid Calibrator and ARK Linezolid Control.

CUSTOMER SERVICE

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2
	Temperature limitation	IVD	*In Vitro* Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 NAME

ARK™ *Linezolid Assay*

2 INTENDED USE

The ARK Linezolid Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of linezolid in human serum on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of linezolid to help ensure appropriate therapy.

3 SUMMARY AND EXPLANATION OF THE TEST

Linezolid (ZYVOX®, Pfizer, Inc.) [(S)-N-[[3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide] is an oxazolidinone derivative with a predominantly bacteriostatic effect against severe infections caused by methicillin- or vancomycin-resistant Gram-positive bacteria.[1]

ZYVOX is indicated in adults and children for the treatment of the following infections caused by susceptible Gram-positive bacteria: Nosocomial pneumonia; Community-acquired pneumonia; Complicated skin and skin structure infections, including diabetic foot infections, without concomitant osteomyelitis; Uncomplicated skin and skin structure infections; Vancomycin-resistant *Enterococcus* faecium infections.[2]

4 PRINCIPLES OF THE PROCEDURE

The ARK Linezolid Assay is a homogeneous immunoassay based on competition between drug in the specimen and linezolid labeled with recombinant enzyme glucose-6-phosphate dehydrogenase (rG6PDH) 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 related 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
5034-0001-00	**ARK Linezolid Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to linezolid, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Linezolid labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), buffer, bovine serum albumin, sodium azide, and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Linezolid 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.** Reagents were stable up to 60 days when stored on-board the instrument based on supporting data.

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

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum is required. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of linezolid. Time of blood draw since last dose should be noted.
- 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.
- Clarified specimens may be stored up to one week at 2 to 8°C. If testing will be delayed more than one week, specimens should be stored frozen (≤ -20°C) up to four weeks prior to being tested. Care should be taken to limit the number of freeze-thaw cycles.
- **Handle all patient specimens as if they were potentially infectious.**

8 PROCEDURE

Materials Provided

ARK Linezolid Assay – REF 5034-0001-00

Materials Required – Provided Separately

ARK Linezolid Calibrator – REF 5034-0002-00
Quality Controls – ARK Linezolid Control – REF 5034-0003-00

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 full calibration (6-point) procedure using the ARK Linezolid Calibrators A, B, C, D, E, and F; test calibrators in duplicate. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

Recalibrate whenever a new lot of reagents is used or as indicated by quality control results (See **Quality Control** below). Acceptable quality control results are needed to validate a new calibration curve. If a new set of reagents with the same lot number is used, validate the system by assaying controls.

A stored calibration curve was effective up to at least 14 days based on supporting data.

Quality Control (QC)

Laboratories should establish QC procedures for the ARK Linezolid Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements. Ensure that the quality control results meet the acceptance criteria before reporting patient results.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol

The measurement range of the ARK Linezolid Assay is 0.75 – 30.00 µg/mL. Specimens containing linezolid in higher concentrations (>30.00 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.

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

9 RESULTS AND EXPECTED VALUES

Report result units as µg/mL or µmol/L. To convert results from µg/mL linezolid to µmol/L linezolid, multiply µg/mL by 2.964. The linezolid 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.

The assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Refer to **Expected Values**.

10 LIMITATIONS OF PROCEDURE

This assay is designed for use with serum; refer to the section **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

Linezolid is the first of the oxazolidinone antibiotics to gain worldwide acceptance for treating severe infections caused by methicillin- or vancomycin- resistant gram positive bacteria, as well as drug resistant tuberculosis. Minimum inhibitory concentrations (MIC_{90}) are reported to be ≤ 2 mg/L for S. aureus, E. faecalis, E. faecium, S. pneumoniae, and coagulase-negative streptococcus isolates.[3] MIC_{90} for M. tuberculosis is reported as 0.5 mg/L.[4]

Treatment efficacy has been linked two pharmacokinetic parameters – the ratio of the daily area under the plasma concentration curve to the minimum inhibitory concentration (AUC_{24}/MIC), and the proportion of time that the plasma concentration is above the minimum inhibitory concentration (%T>MIC) for the organism.[5] Because AUC and trough concentrations are highly correlated, measurements of trough linezolid concentrations (C_{min}) have been used to ensure effective therapy by targeting C_{min} above the known MIC or MIC_{90} (typically 2 mg/L), or by targeting a trough concentration which predicts AUC to be over 80-120 times MIC.[6,7,8]

Adverse effects associated with linezolid treatment limit its use, and include peripheral neuropathy, liver dysfunction, and suppression of bone marrow, leading to anemia, thrombocytopenia, and pancytopenia. The occurrence of these effects correlates with both dose and duration of treatment. Studies suggest adverse events can be minimized by dose management to keep linezolid trough concentrations below 7 mg/L (for treatment duration ≤14 days)[9,10], or below 2 mg/L for the longer treatment durations associated with tuberculosis.[11]

Significant variability in pharmacokinetics has been observed in certain patient groups (impaired creatinine clearance, critically ill patients, neonates, patients on hemodialysis, and patients taking P-glycoprotein inducers, such as levothyroxine).[12,13] Only 50% of hospital patients given the standard dose of 600 mg twice daily achieved concentrations within a target therapeutic range of 2 – 7 mg/L[14], highlighting the importance of TDM for Linezolid.

Linezolid 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 Beckman Coulter AU680 System.

Sensitivity

Limit of Quantitation (LOQ)

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

Criterion	Linezolid (µg/mL)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.002	0.003
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.041	0.071
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD With acceptable recovery and linearity	0.75

Each laboratory is responsible for determining reporting criteria for linezolid 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 Linezolid Assay is 0.75 – 30.00 µg/mL. Specimens containing linezolid in higher concentrations (>30.00 µg/mL) may be assayed by dilution of the specimen into the measurement range for a quantitative result or otherwise reported as detected above the measurement range. Refer to **Section 8 Procedure – Manual Dilution Protocol**.

Recovery

Analytical recovery was performed by adding concentrated linezolid drug into human serum negative for linezolid. A stock concentrate of linezolid in methanol was added volumetrically to human serum negative for linezolid, 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 (%)
0.75	0.82	108.9
1.5	1.5	97.4
3.0	3.0	100.0
4.0	4.1	102.1
8.0	8.2	102.2
12.0	12.0	99.8
18.0	18.3	101.9
24.0	23.0	96.0
28.0	28.0	99.9

Mean percent recovery: 100.9%

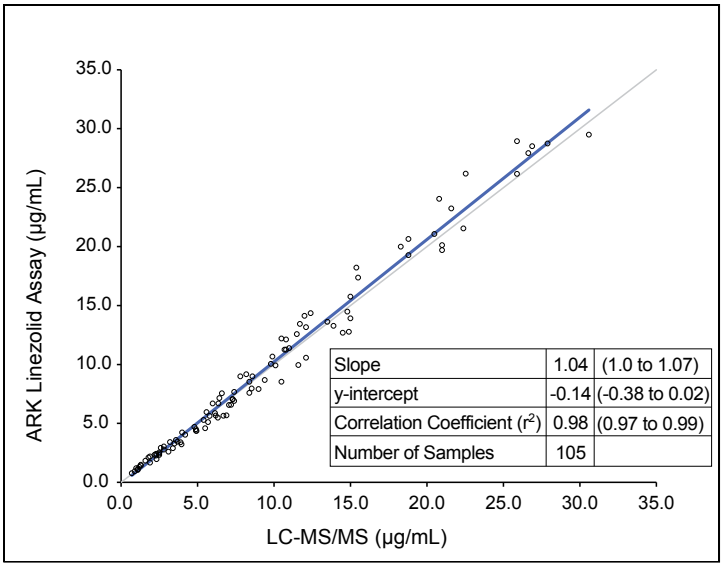
Linearity

Linearity studies were performed as suggested in CLSI EP6-A. A 36.00 µg/mL linezolid serum sample was prepared and dilutions were made proportionally with human serum negative for linezolid. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values, or ±0.20 µg/mL at concentrations ≤1.00 µg/mL. A linear relationship was demonstrated between 0.75 and 30.0 µg/mL (y = 0.9571x + 0.1817).

Nominal (µg/mL)	Measured Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Difference
0.0	0.0	0.18	-0.05	NA
0.75	0.80	0.90	0.72	-0.18 µg/mL
1.5	1.4	1.6	1.5	-8.4%
3.0	2.9	3.1	3.0	-1.6%
6.0	6.0	5.9	6.0	1.5%
9.0	9.0	8.8	9.0	2.1%
12.0	11.8	11.7	11.9	2.0%
15.0	14.9	14.5	14.8	1.7%
18.0	17.4	17.4	17.6	1.2%
21.0	20.7	20.3	20.4	0.6%
24.0	23.6	23.2	23.1	-0.1%
27.0	25.0	26.0	25.8	-0.7%
30.0	28.9	28.9	28.5	-1.4%

Method Comparison

Method comparison studies were performed using CLSI EP09-A3 as a guideline. Results from the ARK Linezolid Assay were compared with results from LC-MS/MS. Passing-Bablok regression analysis was performed for 105 serum specimens with linezolid concentrations by LC-MS/MS that ranged 0.70 µg/mL to 30.6 µg/mL. Passing-Bablok[15] regression statistics are shown below (with 95% confidence limits).



Precision

Precision was determined as described in CLSI EP05-A3. Tri-level controls and three samples of linezolid in pooled human serum 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)	Repeatability Within Run		Between Day		Reproducibility Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Linezolid Control								
LOW	160	2.0	0.08	3.9	0.03	1.7	0.08	4.2
MID	160	10.4	0.41	4.0	0.19	1.9	0.45	4.3
HIGH	160	20.2	0.84	4.2	0.41	2.0	0.93	4.6
Human Serum								
LOW	160	1.9	0.08	4.1	0.04	2.2	0.09	4.6
MID	160	10.6	0.39	3.7	0.15	1.4	0.43	4.0
HIGH	160	20.7	1.03	5.0	0.50	2.4	1.14	5.5

Interfering Substances

Interference studies were conducted using CLSI EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of linezolid (2.0 µg/mL and 10.0 µg/mL) were evaluated. Each sample was assayed using the ARK Linezolid Assay, along with a serum control of linezolid. Measurement of linezolid resulted in ≤10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery (%)	
Interfering Substance	Interferent Concentration	2.0 µg/mL Linezolid	10.0 µg/mL Linezolid
Albumin	12 g/dL	99.8	99.4
Bilirubin - conjugated	72 mg/dL	97.9	95.1
Bilirubin - unconjugated	72 mg/dL	98.5	98.3
Cholesterol	620 mg/dL	103.3	94.5
Human IgG	12 g/dL	98.9	95.2
Hemoglobin	1050 mg/dL	104.3	91.8
Rheumatoid Factor	1080 IU/mL	100.2	105.6
Triglycerides	1670 mg/dL	101.7	102.7
Uric Acid	30 mg/dL	95.6	97.5

Specificity

Metabolism

Linezolid is metabolized in the liver, by oxidation of the morpholine ring, without involvement of the cytochrome P450 system. Clearance of linezolid varies with age and gender; it is fastest in children (which accounts for the shorter half-life), and appears to be 20% lower in women than in men.

Linezolid circulates in plasma mainly as parent drug. Linezolid and two major, inactive metabolites account for the major portion of linezolid disposition, with urinary excretion representing the major elimination route. PNU-142586 accounts approximately 26% of the mean steady-state plasma radioactivity AUC. The secondary metabolite PNU-142300 accounts for approximately 7% of the mean steady-state radioactivity AUC.[16]

Metabolite

The crossreactivity of PNU-142586 linezolid metabolite (100.0 µg/mL) and PNU-142300 linezolid metabolite (100.0 µg/mL) in the ARK Linezolid Assay was not clinically significant (≤ 0.2% crossreactivity). Linezolid (2.0 µg/mL or 10.0 µg/mL in human serum) was tested in the presence of metabolites at higher than expected concentrations of metabolites.

Metabolite (Concentration Tested)	Measured Linezolid in Presence of Metabolite (µg/mL)		
	No Linezolid Present	2.0 µg/mL Linezolid	10.0 µg/mL Linezolid
PNU-142586 (100 µg/mL)	0.0 µg/mL	1.9 µg/mL	10.0 µg/mL
PNU-142300 (100 µg/mL)	0.0 µg/mL	2.0 µg/mL	10.4 µg/mL

Crossreactivity

The compounds listed below did not interfere with the ARK Linezolid Assay when tested in the presence of linezolid (2.0 µg/mL and 10.0 µg/mL). Levels tested were at or above maximum physiological or pharmacological concentrations. Linezolid concentrations of samples containing interferent were compared to the linezolid level in a normal serum control.

Compound	Conc. Tested (µg/mL)	Compound	Conc. Tested (µg/mL)
Acetaminophen	200	Methicillin	250
Acetazolamide	100	Metronidazole	200
Acetylsalicylic acid	1000	Naproxen	600
Amikacin	100	Neomycin	1000
Amitriptyline	20	Niacin	100
Amoxapine	10	Nitrazepam	20
Amphotericin B	100	Nortriptyline	20
Ampicillin	100	Olanzapine	10
Apixaban	10	Oxcarbazepine	100
Ascorbic acid	100	Paroxetine	10
Baclofen	100	Penicillin V	100
Buproprion	10	Perphenazine	100
Caffeine	100	Phenobarbital	200
Chloramphenicol	250	Phenytoin	200
Diazepam	20	Pregabalin	10
Digoxin	10	Procainamide	100
Doxepin	10	Prochlorperazine	10
Edoxaban	10	Ranitidine	100
Erythromycin	200	Rifampin	100
Ethotoin	100	Risperidone	10
Ethosuximide	250	Rivaroxaban	10
Felbamate	250	Sertraline	100
Fluoxetine	10	Spectinomycin	100
Furosemide	100	Stiripentol	100
Gentamicin	100	Sulfamethoxazole	400
Haloperidol	10	Theophylline	200
Ibuprofen	500	Thioridazine	10
Kanamycin A	200	Tobramycin	100
Lamotrigine	200	Trimethoprim	100
Lidocaine	100	Valproic Acid	600
Lincomycin	1000	Vancomycin	100
Meropenem	100	Vigabatrin	150
Mesoridazine	10	Voriconazole	100

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14 TRADEMARKS

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