ARK™ *Lacosamide Assay*

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

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable. A Summary of Safety and Performance is available through Eudamed (European database on medical devices), SRN: US-MF-000023925.

Customer Service

ARK Diagnostics, Inc.

48089 Fremont Blvd
Fremont, CA 94538 USA
Tel: 1-877-869-2320
Fax: 1-510-270-6298
customersupport@ark-tdm.com
www.ark-tdm.com

CE
2797

EC REP

Emergo Europe
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE 2797	CE Mark with notified body number
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2
Rx Only	For Prescription Use Only		

Reagent Kit REF 5033-0001-00

1 Name

ARK™ *Lacosamide Assay*

2 Intended Use

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

3 Summary and Explanation of the Test

Lacosamide (*Vimpat*®, UCB, Inc.) [(R)-2-acetamido-N-benzyl-3-methoxypropionamide] is indicated for the treatment of partial-onset seizures in patients ≥4 years of age, and as adjunctive therapy in the treatment of primary generalized tonic-clonic seizures in patients ≥4 years of age.[1]

4 Principles of the Procedure

ARK Lacosamide Assay is a homogeneous immunoassay based on competition between drug in the specimen and lacosamide 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 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
5033-0001-00	**ARK Lacosamide Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to lacosamide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Lacosamide labeled with bacterial G6PDH, buffer, bovine serum albumin, sodium azide, and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Lacosamide 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 Lacosamide 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. Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- 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

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- Serum is required. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of lacosamide. 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).
- Follow the collection tube manufacturer's recommendations for collection, processing and centrifugation.
- CLSI document GP44-A4 outlines procedures for minimizing artifacts due to specimen collection and handling for common laboratory tests.[13]
- 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.

- The presence of bubbles or foam on specimens can lead to short samples delivery and erroneous results.
- Each laboratory should consult available literature and internal data regarding specimen stability.
-
- 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 Lacosamide Assay – **REF** 5033-0001-00

Materials Required – Provided Separately
ARK Lacosamide Calibrator – **REF** 5033-0002-00
Quality Controls – ARK Lacosamide Control – **REF** 5033-0003-00

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.

Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Lacosamide Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets which have been CLIA categorized or bear the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

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 Lacosamide Calibrators A, B, C, D, E, and F; run 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 Lacosamide 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 Lacosamide Assay is 0.50 – 24.00 µg/mL. Specimens containing lacosamide in higher concentrations (>24.00 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

Report result units as µg/mL or µmol/L. To convert results from µg/mL lacosamide to µmol/L lacosamide, multiply µg/mL by 3.995. The lacosamide 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.

A therapeutic range for lacosamide has not been well established. The reference range of 5 µg/mL to 10 µg/mL[2] or 10 µg/mL to 20 µg/mL[3] has been proposed. *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

Therapeutic drug monitoring of antiepileptic drug (AED) is used worldwide as an aid to individualize drug therapy, and various guidelines have been published that highlight the particular properties of AEDs and the features of epilepsy that make such monitoring so useful.[4-7] A therapeutic range for lacosamide has not been well established. The reference range of 5 µg/mL to 10 µg/mL[2] or 10 µg/mL to 20 µg/mL[3] has been proposed. Steady state concentrations may be achieved after 3 days of treatment.[8] Lacosamide concentrations in serum increased dose dependently, were age independent, and were higher in women compared with men.[9] Coadministration of carbamazepine and phenytoin (inducers of drug-metabolizing enzymes) may decrease serum concentrations of lacosamide substantially.[9,10]

Lacosamide 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 Lacosamide Assay. Analyzer-specific performance may vary.

Criterion	Lacosamide (µg/mL)
Limit of Blank (LoB); N = 60 µB + 1.645 SD, where SD = 0.000	0.000
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.006	0.010
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD With acceptable recovery and linearity	0.40

Each laboratory is responsible for determining reporting criteria for lacosamide 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 analytical measurement range of the ARK Lacosamide Assay is 0.50 – 24.00 µg/mL. Specimens containing lacosamide in higher concentrations (>24.00 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 lacosamide drug into human serum negative for lacosamide. A certified stock concentrate of highly pure lacosamide was added volumetrically to human serum negative for lacosamide, 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 (%)
0.40	0.36	90.4
0.50	0.47	93.3
1.00	1.04	104.2
3.00	3.07	102.3
6.00	6.15	102.6
9.00	8.92	99.1
15.00	14.42	96.1
20.00	21.15	105.8

Mean percent recovery: 99.2

Linearity

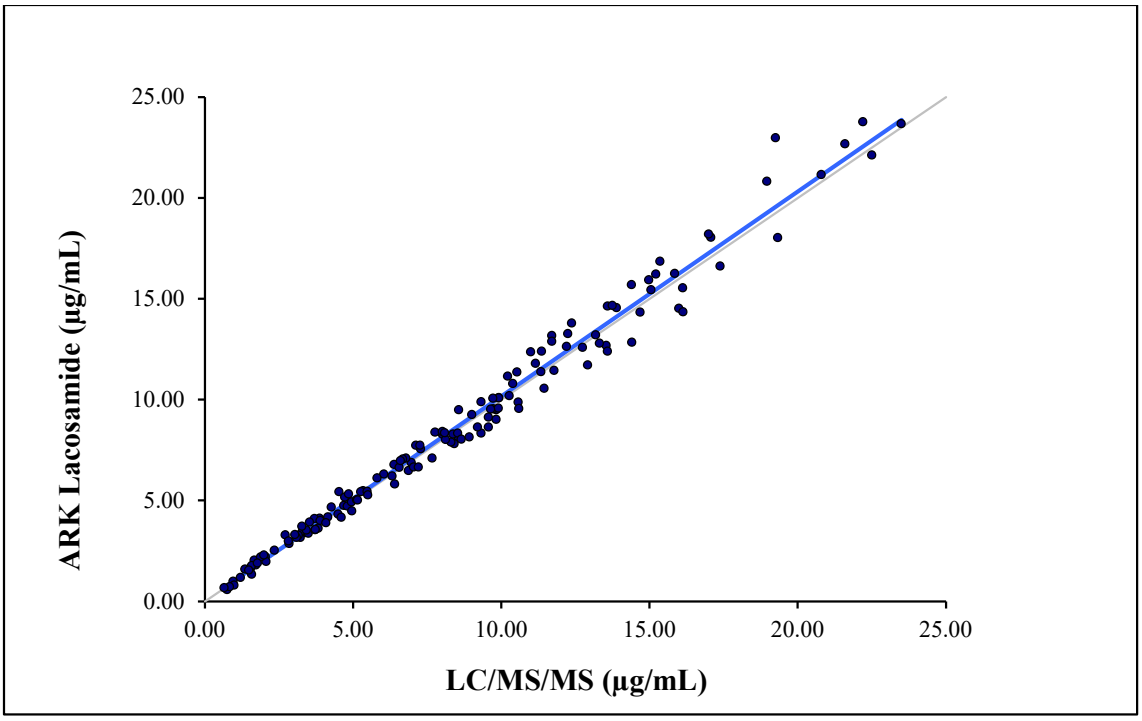
Linearity studies were performed as suggested in CLSI EP6-A. A 30.00 µg/mL lacosamide serum sample was prepared and dilutions were made proportionally with human serum negative for lacosamide. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values, or ≤ 0.20 µg/mL at concentrations ≤ 1.00 µg/mL. A linear relationship was demonstrated between 0.40 and 25.00 µg/mL (y = 0.9998x – 0.0170).

Nominal (µg/mL)	Measured Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Difference
0.00	0.00	-0.02	-0.08	NA
0.40	0.36	0.38	0.33	-0.05 µg/mL
1.50	1.55	1.48	1.45	-2.0 %
3.00	2.95	2.98	2.98	0.0 %
6.00	5.83	5.98	6.02	0.7 %
9.00	8.91	8.98	9.05	0.7 %
12.00	12.01	11.98	12.05	0.6 %
15.00	15.02	14.98	15.04	0.4 %
18.00	18.11	17.98	18.01	0.2 %
21.00	21.41	20.98	20.97	-0.1 %
25.00	24.55	24.98	24.87	-0.4 %

Method Comparison

Method comparison studies were performed using CLSI EP09-A3 as a guideline. Results from the ARK Lacosamide Assay were compared with results from LC-MS/MS. Passing-Bablok regression analysis was performed for 150 serum specimens with lacosamide concentrations by LC-MS/MS that ranged 0.65 µg/mL to 23.50 µg/mL. Passing-Bablok[11] regression statistics are shown below (with 95% confidence limits).

Slope	1.01	(0.99 to 1.04)
y-intercept	0.03	(-0.10 to 0.15)
Correlation Coefficient (r^2)	0.98	(0.98 to 0.99)
Number of Samples	150	



Precision

Precision was determined as described in CLSI EP05-A3. Tri-level controls (1.50, 7.00 and 15.00 µg/mL) and three samples containing equivalent levels of lacosamide 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 Lacosamide Control								
LOW	160	1.55	0.049	3.1	0.049	3.1	0.070	4.5
MID	160	7.13	0.202	2.8	0.204	2.9	0.287	4.0
HIGH	160	14.94	0.450	3.0	0.445	3.0	0.664	4.4
Human Serum								
LOW	160	1.49	0.045	3.0	0.037	2.5	0.058	3.9
MID	160	7.10	0.175	2.5	0.217	3.1	0.283	4.0
HIGH	160	15.18	0.456	3.0	0.432	2.8	0.657	4.3

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 lacosamide (2.0 and 15.0 µg/mL) were evaluated. Each sample was assayed using the ARK Lacosamide Assay, along with a serum control of lacosamide. Measurement of lacosamide resulted in ≤10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery (%)	
Interfering Substance	**Interferent Concentration**	**2.0 µg/mL Lacosamide**	**15.0 µg/mL Lacosamide**
Albumin	12 g/dL	99.8	101.7
Bilirubin - conjugated	70 mg/dL	97.3	96.5
Bilirubin - unconjugated	70 mg/dL	101.1	98.3
Cholesterol	600 mg/dL	95.8	100.1
Gamma-Globulin	12 g/dL	103.5	98.5
Hemoglobin	1000 mg/dL	101.0	101.6
Rheumatoid Factor	1000 IU/mL	97.3	96.8
Triglycerides	1000 mg/dL	97.9	96.2
Uric Acid	30 mg/dL	102.5	96.6

Specificity

Metabolism

Lacosamide is eliminated primarily from the systemic circulation by renal excretion and biotransformation. After oral and intravenous administration, approximately 95% of lacosamide administered was recovered in the urine and less than 0.5% in the feces. The major compounds excreted were unchanged lacosamide (approximately 40% of the dose), its O-desmethyl metabolite (approximately 30%), and a structurally unknown polar fraction (~20%). The plasma exposure of the major human metabolite, O-desmethyl-lacosamide, is approximately 10% of that of lacosamide. This metabolite has no known pharmacological activity.[1,12]

Metabolite

The crossreactivity of O-desmethyl lacosamide metabolite (5.0 µg/mL or 30.0 µg/mL) in the ARK Lacosamide Assay was not clinically significant (≤ 3.0% crossreactivity). Lacosamide (2.0 µg/mL or 15.0 µg/mL in human serum) was tested in the absence or presence of metabolite at higher than expected concentrations of metabolite.

O-Desmethyl Lacosamide (µg/mL)	Measured Lacosamide in Absence/Presence of Metabolite (µg/mL)			
	Lacosamide (2.0 µg/mL)		Lacosamide (15.0 µg/mL)	
	Metabolite Absent	Metabolite Present	Metabolite Absent	Metabolite Present
5.0	2.18	2.23	Not Tested	
30.0	Not Tested		15.51	16.40

Crossreactivity

The compounds listed below did not interfere with the ARK Lacosamide Assay when tested in the presence of lacosamide (2.0 µg/mL and 15.0 µg/mL). Levels tested were at or above maximum physiological or pharmacological concentrations. Lacosamide concentrations of samples containing interferent were compared to the lacosamide level in a normal serum control.

Compound	Conc. Tested (µg/mL)	Compound	Conc. Tested (µg/mL)
Acetaminophen	200	Lincomycin	1000
Acetazolamide	100	Mephenytoin	100
Acetylsalicylic acid	1000	Mesoridazine	10
Amikacin	100	Methicillin	250
Amitriptyline	20	Naproxen	600
Amoxapine	10	Neomycin	1000

Compound	Conc. Tested (µg/mL)	Compound	Conc. Tested (µg/mL)
Amphotericin B	100	Niacin	100
Ampicillin	100	Nitrazepam	20
Ascorbic acid	100	Nortriptyline	20
Baclofen	100	Olanzapine	10
Buproprion	10	Oxcarbazepine	100
Caffeine	100	Paroxetine	10
Carbamazepine	100	2-phenyl-2-ethyl-malonamide (PEMA)	1000
Chloramphenicol	250	Penicillin V	100
Chlorpromazine	10	Perphenazine	100
Citalopram	10	Phenobarbital	200
Clobazam	100	Phenytoin	200
Clonazepam	10	Pregabalin	200
Cyclosporin A	40	Primidone	100
Diazepam	20	Procainamide	100
Digoxin	10	Prochloroperazine	10
Doxepin	10	Ranitidine	100
Erythromycin	200	Rifampin	100
Ethanol	4000 (0.4%)	Risperidone	10
Ethotoin	100	Sertraline	100
Ethosuximide	250	Spectinomycin	100
Felbamate	250	Stiripentol	100
Fluoxetine	10	Sulfamethoxazole	400
Furosemide	100	Theophylline	200
Gentamicin	100	Thioridazine	10
Haloperidol	10	Tobramycin	100
Heparin	200 U/mL	Tiagabine	200
Ibuprofen	500	Topiramate	250
Imipramine	10	Trimethoprim	40
Kanamycin A	200	Valproic Acid	600
Gabapentin	200	Vancomycin	250
Lamotrigine	400	Vigabatrin	150
Levetiracetam	400	Zonisamide	400
Lidocaine	100		

13 References

1. Prescribing information. 2020. VIMPAT® UCB Inc. Smyrna, GA.

2. Kellinghaus, C. 2009. Lacosamide as treatment for partial epilepsy: Mechanisms of action, pharmacology, effects, and safety. Ther Clin Risk Manag **5**:757–766.

3. Greenaway, C. et al. 2010. A high-performance liquid chromatography assay to monitor the new antiepileptic drug lacosamide in patients with epilepsy. Ther Drug Monit **32**:448– 452.

4. Johannessen, S. I. et al. 2003. Therapeutic drug monitoring of the newer antiepileptic drugs. Ther Drug Monit **25**:347–363.

5. Patsalos, P. N. et al. 2008. Antiepileptic drugs—best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE commission on therapeutic strategies. Epilepsia **49**:1239–1276.

6. Krasowski, M. 2010. Therapeutic drug monitoring of the newer anti-epilepsy medications. Pharmaceuticals (Basel) **3**:1909–1935.

7. Brandt, C. and T. W. May. 2011. Therapeutic drug monitoring of newer antiepileptic drugs. J Lab Med **35**:161–169.

8. Chung, S. S. 2010. Lacosamide: new adjunctive treatment option for partial-onset seizures. Expert Opin Pharmacother **11**:1595–1602.

9. Markoula, S. et al. 2014. Lacosamide serum concentrations in adult patients with epilepsy: The Influence of Gender, Age, Dose, and Concomitant Antiepileptic Drugs. Ther Drug Monit **36**:494–498.

10. Contin, M. and F. Albani. 2013. Lacosamide therapeutic monitoring in patients with epilepsy: Effect of concomitant antiepileptic drugs. Ther Drug Monit **35**:849-52.

11. Bablok, W. et al. 1988. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry. Part III. J Clin Chem Clin Biochem **26**:783 – 790.

12. Bialer, M. et al. 2007. Progress report on new antiepileptic drugs: a summary of the eighth Eilat conference (Eilat VIII). Epilepsy Res **73**:1–52.

13. CLSI. *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline—Fourth Edition*. CLSI document GP44-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2010

14 Trademarks

ARK Diagnostics, Inc.
Fremont, CA 94538 USA

Revised March 2025
1600-0391-00 Rev 04