Lamotrigine

Purpose

This procedure provides instructions for performing Lamotrigine on plasma or serum in Children's Minnesota Laboratory.

The ARK Lamotrigine Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of lamotrigine in human serum or plasma on automated clinical chemistry analyzers. The results obtained can be used as an aid in management of patients treated with lamotrigine.

Policy Statements

This procedure applies to all personnel who run the Dimension Vista 500 and Dimension RxL MAX.

Principle

ARK Lamotrigine Assay is a homogeneous enzyme immunoassay based on competition between drug in the specimen and lamotrigine 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 coenyzme NAD functions only with the bacterial enzyme used in the assay.

Clinical Significance

Lamotrigine (LAMICTAL®, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is an anti-convulsant drug approved for use in the treatment of epilepsy and is often prescribed as monotherapy or as one component of a multiple anti-epileptic drug therapy.

In a retrospective review of the records of children and adolescents treated with lamotrigine monotherapy between 2001 and 2006, data was collected including demographics, seizure type, etiology of seizures, age at onset of seizures and at initiation of lamotrigine treatment, number of antiepileptic drugs (AEDs) prior to lamotrigine, dose of lamotrigine, length of follow-up, treatment response, and adverse events. Lamotrigine has been shown to confer broad-spectrum, well-tolerated control of epilepsy. Monotherapy is preferable over polytherapy because of better compliance, fewer adverse events, less interactions, lower teratogenicity and lower cost. The aim of the study was to evaluate the efficacy and safety of lamotrigine monotherapy on seizure control in a cohort of children and adolescents with epilepsy. In conclusion, lamotrigine was effective and well-tolerated as monotherapy in children and adolescents for both focal and generalized epilepsies.[5]

Analyzer

Siemens Dimension Vista 500

Sunquest Test Codes

LAMI Lamotrigine, Lamictal CPT: 80175

Sample

Serum **NO GEL**
Refer to specimen collection procedures for collection of diagnostic blood specimens. Plasma (lithium heparin) is an acceptable specimen type, however, using the same specimen matrix for individual patients is preferred, and therefore serum is the preferred specimen type.

Patient Preparation: A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of Topiramate. Time of blood draw since last dose should be noted.

Minimum volume: 200 µL **Actual Test volume**: 4 µL

Stability: 2-8 °C / 7 days, <-10 °C / 1 month. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C

Rejection criteria: Unlabelled specimens, other than serum or heparinized plasma. Specimens collected using gel separators.

Preparation: Preparation:
1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter.
5. Transfer serum/ plasma or prepared urine to a properly labeled RXL SSC or tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

Materials

Supplies
• Dimension Vista Empty Flex™ Reagent Cartridge available from Siemens PN S999 • Automatic Pipettes and tips • Sample Cups • Sample Segments • 6 mL syringe • 2 mL syringe • 21 gauge needles

Reagents	Stability	Preparation
ARK Lamotrigine Assay Reagent R1 – Antibody/Substrate **5023-0001-00** 1 X 28 mL	**Unopened:** 2–8°C, upright and tightly closed, expiration date printed on the label Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C Improper storage of reagents can affect assay performance	Liquid, ready to use, may be used directly from the refrigerator
Reagent R2–Enzyme Lamotrigine labeled with bacterial G6PDH 1 X 14 mL		
ARK Lamotrigine Calibrator Kit **5023-0002-00**	**Unopened**: 2-8°C, date on vial **Opened**: 2-8°C and tightly capped, 12 months or date on vial	Ready to use. Mix by gentle inversion before dispensing.

Calibration

Assay Range:	0.85 to 40.00 mcg/mL	
Reference Material:	ARK Lamotrigine Calibrators 5023-0002-00	
Suggested Calibration Levels:	A	0.00 mcg/mL
	B	1.00 mcg/mL
	C	3.00 mcg/mL
	D	9.00 mcg/mL
	E	18.00 mcg/mL
	F	40.00 mcg/mL
Calibration Scheme:	Six levels in duplicate. Verify the calibration with 2 levels of QC	
Calibration Frequency:	• Whenever a new lot number of reagents is used • Whenever indicated by quality control results • Whenever required by standard laboratory protocols • Once every 45 days	
Analytical Measuring Range	0.85 to 40.00 mcg/mL The AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range. Further studies are not necessary.	

Risk and Safety

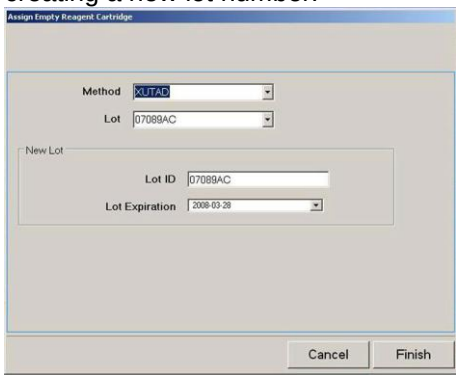
- For *In Vitro Diagnostic* Use. For prescription use only.
- Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Handle all patient specimens as if they were potentially infectious.

Filling the Reagent Flex

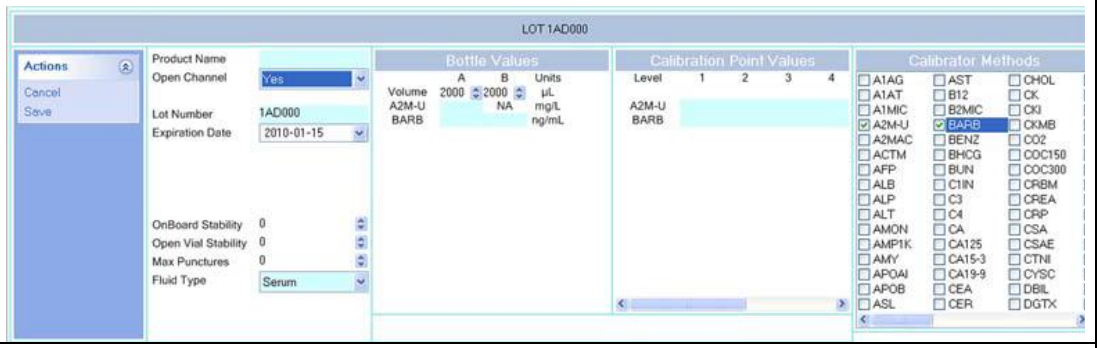
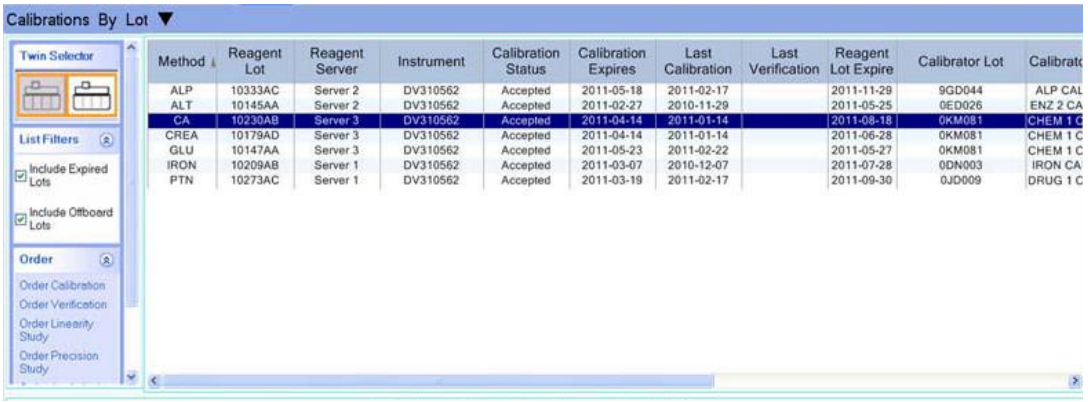
STEP	ACTION
1.	Obtain an empty Dimension Vista Empty Flex™ Reagent Cartridge available from Siemens PN S999 Flex reagent cartridge 
2.	Add Lamotrigine Reagent 1 and Reagent 2 by following steps 3-7
3.	Make a small puncture in the corner of the clear film that covers well 1 and well 12 using a hypodermic needle. Do not remove or tear the film.
4.	Fill a 3 mL syringe with 2.5 mLs of **ARK Lamotrigine Assay Reagent R1 – Antibody/Substrate**, and use the tip of the needle to pierce the opposite corner of the cellophane in well 1.
5.	Fill well 1 with 2.5 mLs of Reagent 1
6.	Fill a new 3 mL syringe with 1.5 mLs of **ARK Lamotrigine Reagent R2–Enzyme** and use the tip of the needle to pierce the opposite corner of the cellophane in well 12.

7.	Fill well 12 with 1.5 mLs of Reagent 2
8.	Load Flex (see procedure below)

Loading of Flex™ Reagent Cartridge

Step	Action
1.	Place the reagent cartridge in the reagent load area and press the **LOAD** button
2.	Press the **Advanced** icon, then the **Inventories** icon
3.	Select the **Reagent Inventory** from the menu
4.	Select the line with EMPTY in the Name column
5.	From the box that appears, select the appropriate test mnemonic for the user-defined method **(XLAM)**
6.	Enter the reagent lot number in the Lot field by selecting the correct lot number, or creating a new lot number. 
7.	In the Lot Expiration field, type the expiration date or select it from the dropdown calendar.
8.	Press **Finish** to accept the data

Calibration

STEP	ACTION
1.	Prepare Calibrators. See Materials section of this procedure.
2.	Program the calibration: Press the **Advanced** icon. Select **Calibration** from the menu
3.	Select **Calibrators** from the menu
4.	Prepare Calibrators. See Materials section of this procedure.
5.	Program the calibration: Press the **Advanced** icon. Select **Calibration** from the menu
6.	Select **Calibrators** from the menu
7.	Select **New** from the bottom of the screen
8.	Select **Yes** from the Open Channel drop-down box
9.	Select the test **(XLAM)** from the check box menu on the side of the screen and enter the bottle values for each calibrator level from the Ark Lamotrigine Calibrator Insert Sheet under the bottle value section 
10.	Select **Save** to finalize Calibrator
11.	From the **Advanced** icon, choose the **Calibration** icon. Select **Calibration by Lot** from the menu 

Calibration (cont)

STEP	ACTION	
12.	Select the XLAM lot needing calibration From the Order menu at the bottom of the screen, select **Order Calibration**. Select the **Use Cups** box for this assay. 	
13.	Verify that the information on the screen is correct. Use the drop-down menu to verify that the calibrator lot is correct, and then press **OK**.	
14.	Mix each calibrator by gentle inversion	
15.	Squeeze 200 uL (about 5 drops) of each of the six calibrators into Vista sample cups	
16.	Place the cups in an adapter into sequential ascending positions in a rack. i.e. calibrator A into position 1, calibrator B into position 2, etc.	
17.	Scan the rack barcode and verify the information on the screen.	
18.	Press **OK** and load the rack on the instrument.	
19.	If all the acceptance criteria are met, the calibration is automatically accepted	
20.	If the acceptance criteria are not met, the status area displays a “Waiting Calibration Review” alert	
21.	Press the **Advanced** icon, then the **Calibration** icon. Select **Calibration by Lot** from the drop down menu	
22.	Select the appropriate method. Review the pending calibration data at the bottom of the screen.	
23.	Enter operator comments if required before pressing **Accept** or **Reject**	
24.	**IF**	**THEN**
	There is an outlier within the replicates	Reject and repeat the calibration
	Quality control fails	Accept the calibration and repeat QC If QC fails repeatedly, perform a new calibration

Quality Control

ARK™ *Lamotrigine Control* 1 X 4 mL Dropper vials
LOW (2.00 mcg/mL)
MID (12.00 mcg/mL)
HIGH (25.00 mcg/mL)
Use each lot as a set

Frequency:

- Two levels of controls must be run every 24 hours
- After loading a new Flex™ reagent cartridge
- After calibration
- After any major maintenance/ repairs have been performed on the analyzer
- When indicated by QC results

Storage and Stability:
Unopened: 2°- 8°C. Use prior to expiration date on container
Open: until expiration date on label when stored tightly capped at 2°- 8°C

Procedure
Controls are ready to use. Mix each level by gentle inversion before dispensing.
Waste 1 drop and then squeeze sufficient volume (~40µL/drop) into individual sample cups.

Sunquest Control names:
Level LOW = C-LAM1
Level MID = C-LAM2

Acceptable Ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.

Interferences

Drug Interference

Interference studies were conducted by Ark using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of potential interfering substances in serum with known levels of lamotrigine (approximately 3 and 15 µg/mL) were evaluated. Each sample was assayed using the ARK Lamotrigine Assay, along with a serum control of lamotrigine. Measurement of lamotrigine resulted in ≤10% error in the presence of interfering substances at the levels tested. See product insert for more information.

Specificity
Lamotrigine's major metabolite, medications that may be routinely co-administered with lamotrigine and other anti-epileptic drugs were tested to determine whether these compounds affect the quantitation of lamotrigine concentrations using the ARK Lamotrigine Assay. High levels of these compounds were spiked into serum pools containing low (3 mcg/mL) and high (15 mcg/mL) therapeutic levels of lamotrigine. The samples were analyzed and the lamotrigine concentrations of samples containing interferent were compared to the serum control.

Metabolites
Lamotrigine is metabolized predominantly by UDP-glucuronyltransferase to form a pharmacologically inactive metabolite, 2-N-glucuronide. Lamotrigine-2-N-methyl has been detected in human plasma by HPLC and capillary electrophoresis. Other minor metabolites, lamotrigine-2-N-oxide, and lamotrigine-5-N-glucuronide have been proposed. Lamotrigine-2- N-glucuronide, Lamotrigine-2-N-methyl and Lamotrigine-2-N-oxide metabolites were tested for cross-reactivity. These metabolites were spiked into two separate samples each containing low and high lamotrigine concentrations of 3 and 15 µg/mL, respectively.

Drug that Cross-Reacts
Cross-reactivity of the antibody to trimethoprim at the following concentration was tested. A high concentration was spiked into normal human serum with known levels of lamotrigine (approximately 3 and 15 **mcg/mL**) and assayed along with a serum control of lamotrigine. The results are shown below.

Interferences (cont)

Trimethoprim (µg/mL)	Percent Cross-Reactivity		Percent Recovery	
	Lamotrigine (3 µg/mL)	Lamotrigine (15 µg/mL)	Lamotrigine (3 µg/mL)	Lamotrigine (15 µg/mL)
40.0	4.4	3.0	156.0	108.0

Care should be taken when interpreting ARK Lamotrigine results if trimethoprim is also being administered to the patient.

Drug Interference
Lamotrigine-selective antibody did not crossreact with most other anti-epileptic or coadministered drugs tested. Due to structural similarities with lamotrigine, high trimethoprim levels may interfere. A high concentration of each compound was spiked into normal human serum with known levels of lamotrigine (approximately 3 and 15 µg/mL) and assayed along with a serum control of lamotrigine. Measurement of lamotrigine resulted in ≤10% error in the presence of drug compounds at the levels tested.

Reference Range

2 to 20 mcg/mL (Medtox)
2.5-15.0 mcg/mL (Mayo by HPLC)
4.0-18.0 mcg/mL (Quest by LC/MS/MS)

A correlation exists between lamotrigine serum level and tolerability, independent of the use of other AEDs. Adverse effects requiring a dose change are uncommon with the most frequently encountered lamotrigine concentrations (<10 mcg/mL) and occur in only 7.4% of patients at levels obtained during the majority of clinical trials (<5 mcg/mL). An initial target range of 1.5 to 10 mcg/mL is suggested, although higher levels, up to more than 20 mcg/mL, are often tolerated and can lead to additional efficacy in refractory patients.[4]

Critical Values

None defined.

Limitations

Linear range of detection: 0.85 to 40.00 **mcg/mL**

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in open channel method results. Refer to your Dimension Vista 500® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments must be resolved prior to reporting.

Dilutions

Above 40.00 **mcg/mL**:

- Dilute results with "assay range" appended.
  - Prepare a 1:2 maximum dilution with the zero calibrator (CAL A), to obtain results within the assay range.
  - Label diluted sample with "label foot" or Accession number, and dilution factor.
  - Program dilution factor (2) in Vista 500 in Manual Data Entry or Special Dilutions. Reassay. Resulting readout is corrected for dilution.
  - Document dilutions and calculations, and have results checked prior to reporting.

Result Reporting

- Results between **0.85 to 40.00 mcg/mL** without error messages are released
- Results below **0.85 mcg/mL**: report as < **0.85 mcg/mL** instead of the numerical value
- Results >**40.00 mcg/mL** are reported as the numerical result following a maximum dilution of 1:2
- Results that exceed the assay range following the maximum dilution are reported as >**80.0 mcg/mL**.
- To convert results from **mcg/mL** (µg/mL) lamotrigine to µmol/L lamotrigine, multiply µg/mL by 3.90

Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

Alternate Method

Refer samples to Medtox Laboratory: test code 158

- When the Children's Hospital method fails quality parameters, and cannot be used
- Twice yearly for Alternate Proficiency assessment, after testing has been completed in house.
- Order test LAMI in Sunquest

References

1. Ark ™ Lamotrigine Assay package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, Printed in USA, Revised October 2010, 1600-0179-00 Rev 02
2. Ark ™ Lamotrigine Calibrator package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, Printed in USA Revised October 2010, 1600-0180-00 Rev 02
3. Ark ™ Lamotrigine Control package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, 1600-0181-00 Rev 02, October 2010
4. Making Sense of Lamotrigine Serum Levels, Bassel W Abou-Khalil, M.D., American Epilepsy Society, Epilepsy Currents, v.5(3); 2005 May, PMC1198624
5. Efficacy and safety of lamotrigine monotherapy in children and adolescents with epilepsy, European Journal of Paediatric Neurology, Volume 13, Issue 2, March 2009, Pages 141–145

Appendices

User Defined Method Specifications

The following specifications are programmed into the Siemens Dimension Vista 500 in the specified field under Advanced -> Configuration -> User Defined Methods. Refer to Dimension Vista 500 Operator's Guide for more help.

Method Name:	ID:	Units:	Mode:	Standard Curve:	Calibration Interval
XLAMO	***2707***	***µg/mL***	***Photometric***	***Logit***	***45 days***

Delivery	Time	Component 1	Remix	Component 2	Chase	Total Volume	Mix
D1	-21	R1 100 µL	None	0	0 µL	100 µL	None
S1	0	S 2.1 µL	None	0	5 µL	7.1 µL	Gentle
D2	82	R2 55 µL	None	0	5 µL	60 µL	Moderate

Cartridge Configuration	Well 1	Well 12
Component:	R1	R2
Tests:	20	20
Well Life [hours]:	72	72
Volume	2250 µL	1305 µL

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	Linda Lichty	January 21, 2016	New test