Methotrexate on Vista 500

Purpose

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

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

Policy Statements

This procedure applies to chemistry staff responsible for analyzing and reporting Methotrexate in serum or plasma on the Dimension Vista 500.

Principle

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate 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

Methotrexate is an anti-neoplastic drug used solely or in combination with other anti-neoplastic drugs for the treatment of leukemia and other diseases. Relatively low doses of methotrexate (7.5 – 25 mg/week) have been used in the treatment of nonmalignant diseases such as severe psoriasis, asthma, rheumatoid arthritis, sarcoidosis, and transplantation therapy. Intermediate to high doses of methotrexate (35 mg/m^2 – 12 g/m^2) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly.

Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors. While the serum level may typically reach 10 to 100 µmol/L, 15 concentrations may exceed 1000 µmol/L with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.

For treatment of osteosarcoma, the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, < 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.

Clinical Significance (cont.)

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs, for example vancomycin. Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite, its molar ratio to methotrexate of up to approximately 100-fold, and relative insolubility versus the parent drug, possible nephrotoxicity due to precipitation of the metabolite in renal tubules may delay elimination of methotrexate itself. Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not the intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.

Analyzer

Siemens Dimension Vista 500, Minneapolis Campus

Sunquest Test Codes

MTX Methotrexate CPT: 80299

Sample

Sample: Heparinized plasma or serum

Minimum volume: 200 µL **Actual sample volume**: 5 µL

- Refer to specimen collection procedures for collection of diagnostic blood specimens
- Use the same specimen matrix for individual patients
- The sampling time of methotrexate is dependent on dose, duration of infusion, and clinical status of the patient. Consult specific Heme/ Onc treatment protocols and Physicians' Desk Reference (PDR) for sampling time information
- Do not induce foaming
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

Stability:
2 to 8°C / two weeks
-20°C /3 freeze-thaw cycles

Rejection criteria: Unlabelled specimens, other than serum or heparinized plasma.

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 may be ultrafuged.
4. Specimens should be free of particulate matter.
5. Transfer plasma/serum to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

Materials

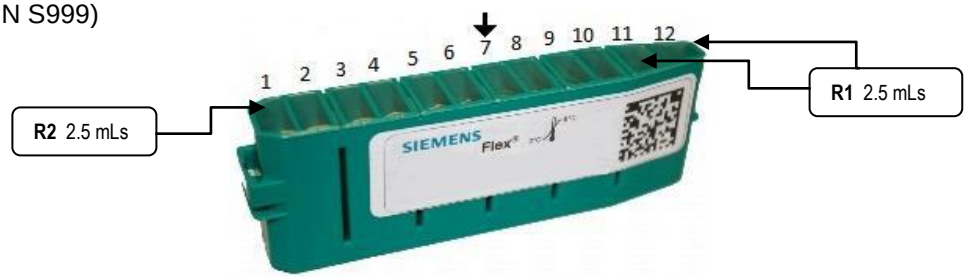
Supplies
• Dimension Vista Empty Flex™ Reagent Cartridge available from Siemens PN S999 • Automatic Pipettes and tips • Sample Cups • Sample Segments • 3 mL syringes • 21 gauge needles

Reagents R1 and R2 need to be transferred to Vista-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2

Reagents	Stability	Preparation
ARK Methotrexate Assay Reagent – Antibody/Substrate R1 **5026-0001-00** 1 X 16 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 Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers.
Reagent – Enzyme R2 **5026-0001-02** 1 X 8 mL		
ARK Methotrexate Calibrator **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.
ARK Methotrexate Calibration Range Controls **25026-0003-01**	**Unopened**: 2-8°C, date on vial **Opened**: 2-8°C and tightly capped, 12 months or date on vial	Controls are ready to use. Mix each level by gentle inversion before dispensing.
ARK Methotrexate Dilution Buffer 5026-0004-00	**Unopened**: 2-8°C, date on vial **Opened**: 2-8°C and tightly capped, 12 months or date on vial	Dilution Buffer is ready to use. Mix by gentle inversion before dispensing. Composition is equivalent to Calibrator A (zero)

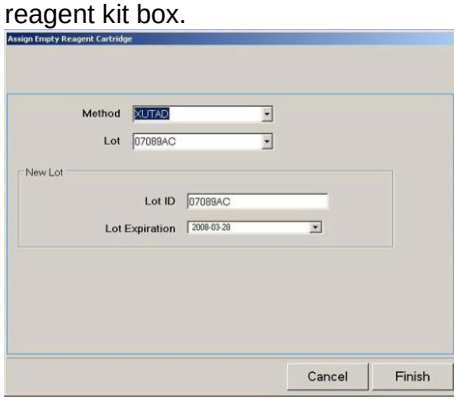
Filling the Reagent Flex

STEP	ACTION
1.	Obtain an empty Dimension Vista Empty Flex™ Reagent Cartridge available from Siemens (PN S999)



2.	Add Methotrexate Reagent 1 and Reagent 2 by following steps 3-8
3.	Make a small puncture in the corner of the clear film that covers wells 1, 11, and 12 using a hypodermic needle. Do not remove or tear the film.
4.	Fill a 3 mL syringe with 2.5 mLs of **ARK Methotrexate Assay Reagent R1 – Antibody/Substrate**, and use the tip of the needle to pierce the opposite corner of the cellophane in well 11.
5.	Fill well 11 with 2.5 mLs of Reagent 1
6.	Repeat steps 4 and 5, adding 2.5 mLs of **ARK Methotrexate Assay Reagent R1 – Antibody/Substrate** to well # 12 also.
7.	Fill a new 3 mL syringe with 2.5 mLs of **ARK Methotrexate Reagent R2–Enzyme** and use the tip of the needle to pierce the opposite corner of the cellophane in well 1.
8.	Fill well 1 with 2.5 mLs of Reagent 2
9.	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 **(XMTX)**
6.	Enter the reagent lot number in the Lot field by selecting the correct lot number, or creating a new lot number when appropriate. Use the lot number on the outside of the reagent kit box. 

7.	In the Lot Expiration field, type the expiration date as it appears on the box, or select it from the dropdown calendar.
8.	Press **Finish** to accept the data

Calibration

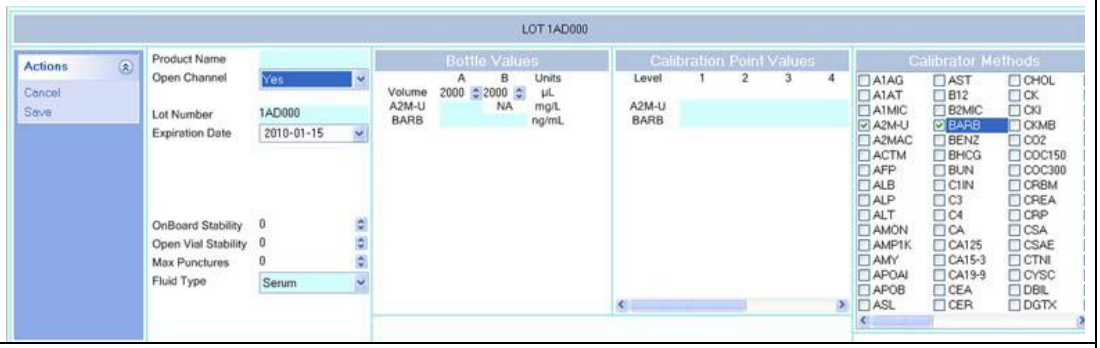
Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E, and F in duplicate.

Assay Range:	0.04 - 1.20 µmol/L.	
Reference Material:	ARK™ Methotrexate Calibrator 5026-0002-00	
Suggested Calibration Levels:	A	0.00 µmol/L
	B	0.05 µmol/L
	C	0.15 µmol/L
	D	0.25 µmol/L
	E	0.50 µmol/L
	F	1.20 µmol/L
Calibration Scheme:	Six levels in duplicate. Verify the calibration with 3 levels of QC Squeeze sufficient volume (about 6 drops) into individual sample cups for each level. Return caps to their original containers and keep tight.	
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	0.04 - 1.20 µmol/L. The AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range. Further studies are not necessary.	

Calibration (cont.)

STEP	ACTION	
1.	Prepare Calibrators. See Materials section of this procedure.	
2.	Program the calibration: Select **System > Method Summary > XMTX** from the methods > **Calibration** > **Order Calibration**	
3.	In the pop-up box that appears verify the reagent lot and calibrator lot are correct: 	
4.	Check that the “Use Cups” box is checked	
5.	Mix each calibrator by gentle inversion	
6.	Waste 1 drop, then squeeze 200 uL (about 5 drops) of each of the six calibrators into Vista sample cups	
7.	Place the cups in an adapter rack in sequential ascending positions in a rack. i.e. calibrator A into position 1, calibrator B into position 2, etc.	
8.	Scan the rack barcode and verify the information on the screen.	
9.	Press **OK** and load the rack in the Reagent Loader Lane	
10.	If all the acceptance criteria are met, the calibration is automatically accepted	
11.	If the acceptance criteria are not met, the status area displays a “Waiting Calibration Review” alert	
12.	Press the **Advanced** icon, then the **Calibration** icon. Select **Calibration by Lot** from the drop down menu	
13.	Select the appropriate method. Review the pending calibration data at the bottom of the screen.	
14.	Enter operator comments if required before pressing **Accept** or **Reject**	
15.	**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

Add a New Lot of Calibrators

STEP	ACTION
1.	Press the **Advanced** icon. Select **Calibration** from the menu
2.	Select **Calibrators** from the menu
3.	Select **New** from the bottom of the screen
4.	Select **Yes** from the Open Channel drop-down box
5.	Select the test **(XMTX)** from the check box menu on the side of the screen and enter the bottle values for each calibrator level from the Ark Methotrexate Calibrator Insert Sheet under the bottle value section 
6.	Select **Save** to finalize Calibrator

Quality Control

ARK Methotrexate Control (2 mL) vials
LOW (0.07 µmol/L)
MID (0.40 µmol/L)
HIGH (0.80 µmol/L)
Use each lot as a set

Frequency:

- Three 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

STEP	ACTION
1.	Press the alert to display the **Needs > QC Vials** screen.
2.	Select the product lot needed, and press the **Print Vial Barcodes** button to print QC vial barcodes.
3.	Controls are ready to use. Mix each level by gentle inversion before dispensing.
4.	Apply the vial labels to the appropriate vials
5.	Transfer sufficient volume (1000µL or 12 drops) into Siemens empty Vista QC vials.
6.	Tighten the cap on the vial. The septum should be flush with the cap. Do not over tighten.
7.	Place the QC vials in a carrier. Make sure the barcode labels are visible through the carrier slots
8.	Place the carrier in the loading area, positioned with the labels facing away from the operator. Press the **Load** button.

Sunquest Control names:
Level LOW = C-MTXL
Level MID = C-MTXM
Level HIGH = C-MTXH

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

Interferences

Interference

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of **methotrexate was not substantially affected** at the levels of endogenous substances tested.(Albumin, Bilirubin – conjugated, Bilirubin – unconjugated, Cholesterol, Gamma-Globulin, Hemoglobin, Intralipid®, Rheumatoid Factor, Triglycerides, Uric Acid)

Specificity

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

- The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.
- After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 12 to 48 hours after HDMTX administration.

Crossreactivity to 2,4-Diamino-N10-methylpteroic acid (DAMPA)
As a minor metabolite of methotrexate, DAMPA is not expected to circulate at concentrations that would interfere in measurement of methotrexate. However, following glucarpidase rescue therapy, the serum concentration of DAMPA can be substantial. The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. ***The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy that rapidly converts circulating methotrexate to DAMPA.***
DAMPA crossreacts with the methotrexate antibody used in this assay, and may continue to circulate for at least 5 - 7 days before accurate measurements of serum methotrexate may return. Oncologists on the clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA.

Drugs that crossreact
The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for MTX cancer treatment due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

Note: glucarpidase (carboxypeptidase G2) is administered rarely at Children's in the event of renal failure. Pharmacy is aware of the cross-reactivity. Triamterene has not been available at Children's, and trimethoprim is contraindicated with methotrexate therapy, and is discontinued prior to methotrexate treatment.

Reference Range

None established.
Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.

Clinical Situation	Laboratory Findings	
	Methotrexate Level (µmol/L)	Hours after administration
Normal Methotrexate Elimination	~10	24
	~1	48
	<0.2	72
Delayed Late Methotrexate Elimination	>0.2	72
	>0.05	96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50	24
	≥5	48
	OR	
	≥100% increase in serum creatinine	24

Critical Values

>1.0 µmol/L Critical values must be called according to the Critical Limit Reporting Policy

Limitations

Linear range of detection: 0.04 - 1.20 µmol/L.

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 1.20 µmol/L:

XMTX on Serum/Plasma	
Maximum Dilution	1:1000
Surplus Rack	Samples with results >1.20 µmol/L are automatically repeated on a higher dilution.
Limited Rack	Samples with results >1.20 µmol/L should be repeated as an Add-On Test with a Special Dilution of 1:100

Manual Dilution:

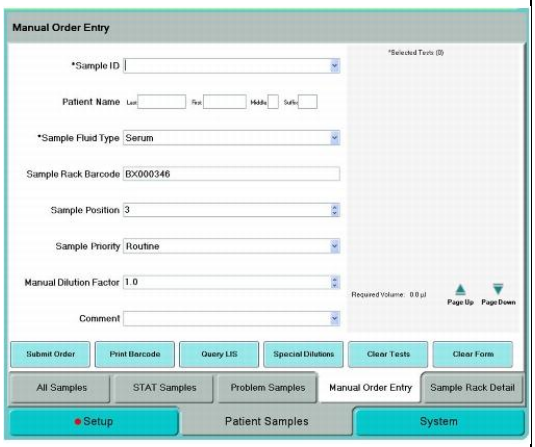
STEP	INSTRUCTIONS
1.	Manually dilute the high specimen or control with **ARK Methotrexate Dilution Buffer** by preparing the appropriate ten-fold serial dilution as shown below.

Sample	Volume	Dilution Buffer Volume	Dilution	Dilution Factor
Undiluted	50 µL	450 µL	1:10	10
1:10 sample	50 µL	450 µL	1:100	100
1:100 sample	50 µL	450 µL	1:1000	1000

Refer to the procedure CH 2.03 Dilution Preparation.

The maximum dilution to prepare is a 1:1000

2.	Go to the Manual Order Entry screen on the Dimension Vista.
3.	Program all samples using the SAMPLE ID preceded by the DILUTION FACTOR to prevent autofiling.
4.	Select test XMTX
5.	Select PRINT BARCODE
6.	Select SUBMIT ORDER
7.	Prepare the dilutions in SAMPLE CUPS. Use the printed barcodes to label your samples.



8.	Place the barcoded sample cup(s) onto a sample rack with teal inserts (NO PILOT TUBE).
9.	Evaluate results of all samples in the serial dilution as a quality check. Compare final results of each dilution with the lowest dilution giving no error message. Correctly prepared dilutions should match within 20% of each other.
10.	Record manual dilution on the dilution log, and result using the Result Reporting section below. Have your dilutions checked by a second tech PRIOR to reporting results.

Result Reporting

- Results between **0.04 - 1.20** µmol/L without error messages are released
- Results below **0.04** µmol/L: report as < **0.04** µmol/L instead of the numerical value
- Results >**1.20** µmol/L are reported as the numerical result following a maximum dilution of 1:1000
- Results that exceed the assay range following the maximum dilution are reported as >**1200.0** µmol/L
- To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005

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 Abbott Laboratory when Children's Hospital method fails quality parameters
- Order test TREX in Sunquest

References

1. ARK™ *Methotrexate* Assay package Insert**,** Revised October 2014 1600-0213-00 Rev 05, 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
2. ARK™ Methotrexate Calibrator Insert, 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
3. ARK™ Methotrexate Control Package Insert, 1600-0215-00 Rev 04 , Revised November 2014, 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
4. Jacobs & DeMott Laboratory Test Handbook, 5th Edition, Lexi-Comp, Inc., Hudson, OH, 2001

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
XMTX	***2710***	***µmol/L***	***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 6.0 µL	None	0	5 µL	11 µL	Gentle
D2	82	R2 50 µL	None	0	5 µL	55 µL	Moderate

Cartridge Configuration	Well 1	Well 11	Well 12
Component:	R2	R1	R1
Tests:	40	20	20
Well Life [hours]:	72	72	72
Volume	2270 µL	2250 µL	2250 µL

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Linda Lichty	June 24, 2016	New method
2.			