| **Alanine Aminotransferase (ALT)** |
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| **Purpose** | This procedure provides instructions for ALANINE AMINOTRANSFERASE (ALT). The alanine aminotransferase (ALTI) method is an *in vitro* diagnostic test for the quantitative measurement of alanine aminotransferase activity in human serum or plasma on the Dimension Vista® 500 or RxL Chemistry Systems. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Dimension Vista® or RxL Chemistry Systems at Children’s Hospitals and Clinics of Minnesota. |
| **Principle** | The alanine aminotransferase method is an adaptation of the recommended procedure of the IFCC as described by Bergmeyer. The procedure is based on the principles outlined by Wroblewski and LaDue but is modified to contain pyridoxal-5-phosphate (P5P) as an activator and to replace phosphate buffer with tris (hydroxymethyl) aminomethane.Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine to a-ketoglutarate (a-KG), forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a bichromatic (340, 700 nm) rate technique. |
| **Clinical Significance** | Measurements of alanine aminotransferase are used in the diagnosis and treatment of certain liver diseases and heart diseases. Significant elevations are found in diseases of the liver, such as hepatitis, necrosis, jaundice and cirrhosis. Alanine aminotransferase levels can be elevated even before clinical jaundice appears. ALT is more sensitive to cellular injury than biliary obstruction, and is more specific for liver injury than AST. Chronic increases occur in alcohol abuse, chronic hepatitis B and C, steatohepatitis, cirrhosis, hemochromatosis, and viral hepatitis. |
| **Instrument** | **PRIMARY METHOD:** Siemens Dimension Vista 500, Siemens Healthcare Diagnostics Inc**SECONDARY (BACKUP) METHOD:** Siemens Dimension RxL MAX  |
| **Sunquest Test Code** | **ALT**: Alanine Aminotransferase in serum or plasma |
| **Specimen** | Plasma (lithium heparin) preferred or Serum. In the preparation of serum or plasma samples, avoid prolonged contact with separated red cells.**Minimum volume:** 200 µL preferred, 100 µL minimum **Actual Test Volume:** 14.6 µL (35 µL secondary method)**Stability:** 2-8 °C / 7 days, < -20°C / 1 month (centrifuge thawed samples prior to use). Avoid repeated freeze thaw cycles.**Rejection criteria:** Unlabeled tube, other than heparinized plasma, or serum**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 or plasma 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.
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| **Reagents** | **PRIMARY METHOD:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| ALTI Flex® reagent cartridge | K2143 | **Store at:** 2 – 8 °C**Unopened:** Date on carton.**On-board:** Sealed wells on the instrument are stable for 30 days.**Open Well Stability:** 10 days for wells 1–2, 11 – 12, 5 days for wells 3 - 10 |
| ENZ 2 CAL – ready for useStandardized IFCC at 37 ° C primary reference method | KC321 | **Store at:** 2 – 8 °C**Unopened:** Date on carton.**On board:** 7 days**Opened:** 30 days recapped and stored at 2 – 8 °C. Do not use this vial on board. |

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| **Reagents (cont)** | **SECONDARY METHOD:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| ALTI Flex® reagent cartridge | DF143 | **Store at:** 2 – 8 °C**Unopened:** Date on carton.**On-board:** Sealed wells on the instrument are stable for 30 days.**Open well stability:** 3 days for wells 1 – 6, 30 days for wells 7-8 |
| Enzyme II Calibrator – ready to use. | DC143 | **Store at** 2 – 8°C**Unopened:** Date on carton.**Opened:** Assigned values are stable for 30 days, capped and stored at 2 – 8°C |
| Enzyme Diluent  | 790035901 | **Store at** 2 – 8°C**Unopened:** Date on carton.**Reconstituted:** Discard after 7 days |

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| **Risk and Safety:** | Harmful. Irritant. Contains 2-chloroacetamideAvoid contact with skinMay cause sensitization by skin contactWear gloves.Safety data sheets (MSDS/SDS) available on [www.siemens.com/diagnostics](http://www.siemens.com/diagnostics) and Children’s Intranet |
| Calibration/ Verification/AMR | **PRIMARY METHOD:**

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| Analytical Measuring Range: | 6–1000 U/L |
| Reference Material: | ENZ 2 CAL, Cat. No. KC321 |
| Suggested Calibration Levels | Level 1 (Calibrator A): 0 U/L Level 2 (Calibrator B): 1050 U/L |
| Verification Scheme: | 2 levels, n=5 |
| Verification Frequency: | * Every 90 days for any one lot.
* For each new lot of Flex® reagent cartridges
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| AMR | Verification of AMR is accomplished with each calibration.* Cal Verification and AMR verification are performed at least once every six (6) months.
* Touch Advanced 🡪 Calibrations 🡪 Calibrations by Lot, select method ALTI and “Order a Linearity Study”
* See iGuide “Calibration by Lot” for more information.
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| Calibration/ Verification/AMR | **SECONDARY METHOD:**

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| Analytical Measuring Range: | 6–1000 U/L |
| Reference Material: | Enzyme II Calibrator (DC143) |
| Suggested Calibration Levels | Level 1: 0 U/L Level 2: 500 U/L Level 3: 1050 U/L  |
| Verification Scheme: | 3 levels, n = 3 |
| Verification Frequency: | * Every 90 days for any one lot.
* For each new lot of Flex® reagent cartridges
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| AMR | Verification of AMR is accomplished with each calibration using 3 calibrators that span the reportable range. Additional studies are not required. |

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| **Quality Control** | **PRIMARY METHOD:** BioRad Mulitqual® 1 & 3 Control Levels, contained in Vista vials**Frequency:** Two levels each day of use**Stability:** Stable until the date on vial when stored at -20 to -50 C protect from light. Thawed and unopened, 7 days at 2 -8 C. 7 days on board the Vista, and 5 days opened and stored at 2 – 8 C**Preparation**: Allow the control to stand at 18 – 25 C for 30 minutes until completely thawed. Gently mix the vials until homogeneous to dissolve any precipitate.**Sunquest Control names:** Level 1 = C-MQ1, Level 2 = C-MQ3**SECONDARY METHOD**: Biorad Liquichek™ Unassayed Chemistry Control (Human) Levels 1 & 2**Frequency:** Two levels each day of use**Stability:** Refer to the current lot product insert**Sunquest Control names:** Level 1 = C-X1, Level 2 = C-X2**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until the cause of deviation has been identified and corrected
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
* When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
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| **Interferences** | Hemolysis, Icterus & Lipemia (HIL) Index Values:

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* Bilirubin (conjugated) at 40 mg/dL decreases ALTI results at an activity of 70 U/L by -12%.
* Bilirubin (conjugated) at 60 mg/dL decreases ALTI results at an activity of 144 U/L by (-) 13%.
* Bilirubin (unconjugated) at 60 mg/dL decreases ALTI results on the **SECONDARY METHOD** at an activity of 68 U/L by -11%.
* Hemolysis of 1000 mg/dL had <10% bias on ALTI results
* Lipemia (Intralipid®) of 600 mg/dL and above tripped a test report message; therefore the magnitude of the interference could not be determined.
* Triglyceride above 400 mg/dL tripped a test report message; therefore the magnitude of the interference could not be determined.
* Refer to the Operator’s Guide for follow up on specific test report messages
* **PRIMARY METHOD: The drugs Sulfasalazine and Sulfapyridine interfere** **with this assay**. Venipuncture should occur prior to sulfasalazine and/or sulfapyridine administration due to the potential for falsely depressed results.
* **SECONDARY METHOD: The drug Sulfasalazine interferes with this assay.** Venipuncture should occur prior to sulfasalazine administration due to the potential for falsely depressed results.
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| **Reference Range** | 6-50 U/L |
| **Critical Values** | None specified |
| **Limitations** | The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to the iGuide or Dimension® Operator’s Guide for troubleshooting specific error messages. |
| **Dilutions** | **PRIMARY METHOD:** **Serum/Plasma**

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| Maximum Dilution: | 1:20 |
| Surplus Rack: | Samples with results >1000 U/L reflex to higher dilutions (1:3.51, 1:10, 1:20).  |
| Limited Rack: | Samples with results >1000 U/L should be repeated as an Add-On Test with the lowest dilution (1:3.51, 1:10, or 1:20) that will give a result without an error message.  |
| Manual Dilution | Do not manually dilute. |

**SECONDARY METHOD**:**Above 1000 U/L:** The instrument makes an autodilution.* Results with “dilution” appended are reportable.
* Dilute results with “assay range / dilution” appended.
* Make appropriate dilution with Enzyme Diluent (PN 790035901) or equivalent to obtain results within assay range.
	+ Determine optimum dilution by dividing RxL result obtained by 1000 (the assay range) and rounding up to the next whole number.
	+ The maximum dilution to prepare is 1:20
* Label diluted sample with “label foot” or Accession number, and dilution factor.
* Program dilution factor. Reassay. Resulting readout is corrected for dilution.
* Document dilutions and calculations, and have results checked prior to reporting.
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| **Result Reporting** | * Results between 6 - 1000 U/L without error messages are released
* Results less than 6 are reported as < 6 rather than the numerical value
* Results >1000 U/L without error messages are reported following a maximum dilution of 1:20
* Results with “assay range” appended following a maximum dilution of 1:20 are reported as >20,000 U/L
* Append the comment “BIN” to icteric samples with a HIL index of 7 or greater.
* Append the comment “LINT” to lipemic samples with a HIL index of 4 or greater.
* Refer to [CH5.101 HIL on Dimension Vista](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Procedure/206820.pdf)
* Results with “Abnormal assay” are reported as METH. Do not report a numerical value
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| **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. |
| **References** | 1. Vista ALTI Flex™ reagent cartridge insert sheet Siemens Healthcare Diagnostics Inc., PN 781143.001 version D, Issue Date 2015-02-06
2. ALTI Flex™ reagent cartridge insert sheet Siemens Healthcare Diagnostics Inc., PN 717143.002 – US, version F Issue Date 2015-01-30
3. Dimension Vista Enzyme 2 Calibrator insert sheet, Siemens Healthcare Diagnostics Inc., PN 751321.001-US, Rev A, 01/2011
4. Dimension Clinical Chemistry System Enzyme II Calibrator insert sheet, Siemens Healthcare Diagnostics Inc., PN 792143.001 version A, issue date 02-2011
5. Clinical Significance, Dade Behring Inc., Glasgow Business Community, Mailbox 531, P.O. Box 6101, Newark, Delaware 19714
6. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001
7. Biorad Multiqual Assayed Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
8. Biorad Liquichek Unassayed Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
9. Siemens Urgent Medical Device Correction CHC-17-06.A.US.DM June 29, 2017
10. [CLASS kids reference range](http://www.classkids.org/pediatric-lab-values.html)
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| **Historical****Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | D. Riedel | Nov. 2000 | Initial Version |
|  | L. Lichty | 6/5/2005 |  |
|  | L. Lichty | 12/22/06 |  |
|  | D. Helfinstine | April 1, 2011 | Package insert revisions, new format, renumbered from CH 3.08 |
|  | L. Lichty | 2/28/13 | Clarify max dilution reporting |
|  | L. Lichty | 12/17/2013 | Siemens ALTI CLSI – Dimension Vista – Rev C, 7/17/2013  |
|  | L. Lichty | 12/19/13 | Revised reagent on RxL |
|  | L. Lichty | 5/21/2015 | Updated for Vista |
|  |  | K. Brown/S. Gripentrog | June 1, 2017 | Removed no gel from serum sample requirements. Updated product insert issue date. |
|  |  | Kelsi Brown | July 17, 2017 | Updated interfering substances after notification from Siemens. |
|  |  | Erin Bartos | August 1, 2017 | Added references for interfering substances and new reference range and changed reference range from 30-65 U/L to 6-50 U/L |
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