| **Ammonia (NH3)** | | | | |
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| **Purpose** | This procedure provides instructions for performing the AMM method, an *in vitro* diagnostic test for the quantitative measurement of AMMONIA (NH3) in plasma on the Dimension Vista® System. | | | |
| **Policy Statements** | This procedure applies to all personnel who run the Siemens Dimension Vista. | | | |
| **Principle** | The Dimension Ammonia (AMM) assay is an enzymatic method that uses glutamate dehydrogenase (GLDH) and a stabilized NADPH analog. Ammonia reacts with a-ketoglutarate and reduced cofactor to form L-glutamate and the cofactor. The reaction is catalyzed by glutamate dehydrogenase. The decrease in absorbance due to the oxidation of the reduced cofactor is monitored at 340/700 nm and is proportional to the ammonia concentration. | | | |
| **Clinical Significance** | Ammonia measurements are used in the diagnosis and treatment of severe liver disorders such as cirrhosis, hepatitis and Reye’s syndrome. As a rule, only elevated levels of blood ammonia are useful in diagnosis. Ammonia is produced by nearly all body cells in which amino acids are degraded. The major source of circulating ammonia is the gastrointestinal (GI) tract. Under normal conditions, ammonia is metabolized to urea by liver enzymes. Several diseases, both inherited and acquired, cause elevated ammonia (hyperammonemia). The inherited deficiencies of urea cycle enzymes are the major cause of hyperammonemia in infants. Acquired hyperammonemia most often results from liver disease, renal failure, and Reye’s syndrome. Elevated ammonia is toxic to the central nervous system. | | | |
| **Analyzer** | **PRIMARY METHOD:** Siemens Dimension Vista® 500 System  **SECONDARY (BACKUP) METHOD:** Siemens Dimension Vista® 500 on opposite campus, freeze sample @ -70 °C prior to transport, and transport on dry ice. | | | |
| **Sunquest Test Code** | **NH3** - Ammonia in plasma in µmol/L | | | |
| **Specimen** | **Patient Preparation:** The patient should avoid smoking for 1 hour prior to specimen collection.  **Sample:**   * Lithium Heparinized plasma collected by venipuncture. * Transport specimen to lab on ice slurry within 20 minutes of collection. * The tube should be completely filled, and immediately stored tightly capped on ice,   **Minimum volume:**  200 µL, preferred, 100 µL minimum, 20 µL actual test volume.  **Stability:**   * Analyze within 30 minutes of centrifugation. * 2 hours at 2 – 8 C once separated. Concentrations may more than double in plasma when stored at room temperature for 6 hours. * -70 °C for 2 days once separated if testing must be delayed. | | | |
| **Specimen (cont)** | **Rejection criteria:**   * Unlabeled tube * Clotted Samples * Not received on ice within 20 minutes of collection, * Hemolyzed samples, HIL index of >3 * Fingerstick collection * Lipemic samples, Lipemia (Intralipid®) of >100 mg/dL that trips an error message   **Preparation**   1. Plasma should be physically separated from cells immediately. Specimens should be free of particulate matter. See processing procedure manual. 2. 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. 3. Analyze sample immediately, or store appropriately. See above. | | | |
| **Reagents** | |  |  |  | | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | AMM Flex Reagent Cartridge (Vista) | K3119 | **Store at:** 2 - 8 °C.  **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges.  **On-board:** Sealed wells on the instrument are stable for 60 days.  **Open well stability:** 3 days for wells 1 – 12 | | CHEM 3 CAL | KC130A | **Store at:** 2 - 8 °C.  **Unopened:** Refer to carton for expiration date.  **On-board:** Once the vial stopper is punctured, assigned values are stable for 24 hours when stored on board the Dimension Vista System  **Opened**: Assigned values are stable for 30 days when recapped immediately and stored at 2 - 8 °C. Do not use this vial on board. | | | | |
| **Risk and Safety** | Irritant. Contains a mixture of 5-chloro-2-methyl-2H-isothiazol -3-one and 2‑methyl‑2H‑isothiazol-3-one (3:1).   * May cause sensitization by skin contact. * Avoid contact with skin. * Wear suitable gloves. * Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics * Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion. | | | |
| **Calibration** | |  |  | | --- | --- | | Assay Range: | 10 - 750 µmol/L | | Reference Material: | CHEM 3 CAL (Cat. No. KC130A) | | Typical Calibration Levels: | Level 1 (Calibrator A): 0 µmol/L  Level 2 (Calibrator B): 850 µmol/L | | Calibration Scheme: | Two levels, n=5 | | Calibration Frequency: | * For each new lot of Flex® reagent cartridges * Every 60 days for any one lot. * After major maintenance or service, if indicated by QC results * As indicated in laboratory quality control procedures * When required by government regulations | | | Sample Size: | Standard sample size = 20 µL  Autodilute sample size = 10 µL @ 1:2 | | Analytical Measuring Range | 10-750 µmol/L | | | | |
| **Analytical Measuring Range (AMR)** | * Cal Verification and AMR verification are performed at least once every six (6) months. * Touch Advanced 🡪 Calibrations 🡪 Calibrations by Lot, select method AMM and “Order a Linearity Study” * See iGuide “Calibration by Lot” for more information. | | | |
| **Quality Control** | Bio-Rad Liquichek™ Ethanol/Ammonia Control, Level 1 & 3 supplied in Vista Vials  **Frequency:** Daily  **Stability:** Refer to the current lot product insert  **Sunquest Control names:** Level 1 = C-NHL, Level 3 = C-NHH  **Acceptable ranges:**   * Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. * If a control value is outside the confidence interval, the determination must be repeated. If the repeated 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 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 | | | |
| **Interferences** | Hemolysis, Icterus & Lipemia (HIL) Index Values:  **DO NOT USE HEMOLYZED SAMPLES!**   |  |  |  | | --- | --- | --- | | **H** | **I** | **L** | | 4 | 7 | 3 |   Interfering Substances:   * Hemolyzed samples may indicate traumatic collection. Hemoglobin increases AMM results. **DO NOT USE HEMOLYZED SAMPLES.** * Smoking, laboratory atmosphere, venipuncture technique, and in vitro amino acid metabolism are all possible sources of error for the measurement of ammonia. * Bilirubin (unconjugated) of 60 mg/dL decreases AMM results by 16% at an ammonia concentration of 50 μmol/L, and decreases AMM results by <10% at an ammonia concentration of 426 μmol/L. * Dextran increases ammonia results. Refer to product insert for additional information. * Elevated IgG may increase AMM results. Refer to product insert for more information. * Lipemia (Intralipid®) of >100 mg/dL tripped a test report message; therefore the magnitude of the interference could not be determined. **Do not clear lipemia** in samples that tripped a test report message, as the results may be invalid. * Triglycerides at 3000 mg/dL tripped a test report message; therefore the magnitude of the interference could not be determined. * Sulfasalazine and Sulfapyridine interfere with NADH and or/NADPH reaction assays. Venipuncture should occure prior to sulfasalazine administration due to the potential for falsely elevated results. Venipuncture should occure prior to sulfasalazine administration due to the potential for falsely depressed results.   Refer to the Siemens IFU for a list of non-interfering substances. | | | |
| **Reference Range** | |  |  | | --- | --- | |  | | | AGE | NORMAL | | 18 years | 11-32 µmol/L | | 1 month | 21-50 µmol/L | | 14 days | 56-92 µmol/L | | 0 days | 64-107 µmol/L | |  | | | | | |
| **Critical Values** | >150 µmol/L. Call result according to Critical Results Reporting Policy | | | |
| **Limitations** | Linear range of detection: 10 - 750 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 results. Refer to the Dimension Vista® iGuide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed and not reported. | | | |
| **Dilutions** | |  |  | | --- | --- | | Maximum Dilution: | 1:2 | | Surplus Rack: | Samples with results >750 µmol/L reflex to a 1:2 autodilution. | | Limited Rack: | Samples with results >750 µmol/L should be repeated as an Add-On Test with a Special Dilution of 1:2. | | Manual Dilution | Do not dilute manually. |   Results with “dilution” appended are reportable. | | | |
| **Result Reporting** | * Results between 10 - 750 µmol/L without error messages are released. * Results below 10 µmol/L are reported as <10 µmol/L. * Results > 750 µmol/L without error messages following a maximum dilution of 1:2 may be reported. * Results with “Above assay range” appended following a maximum dilution of 1:2 are reported as > 1500 µmol/L * Append the comment “BIN” to samples with a HIL index of 7 or greater. Refer to [CH5.101 HIL on Dimension Vista](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Procedure/206820.pdf) | | | |
| **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. Siemens Dimension Vista® AMM Flex® reagent cartridge Instructions for Use, Siemens Healthcare Diagnostics, PN 781119.001 Issue Date 04-08-2013 Rev. A 2. Siemens Dimension Vista® Chem 3 Cal Instructions for Use, Siemens Healthcare Diagnostics, June 1, 2014 3. Burtis, CA and Ashwood, ER, Tietz Textbook of Clinical Chemistry, 3rd Edition, Philadelphia, PA: WB Saunders Co. 1999, p 1146-7. 4. Biorad Liquichek Ethanol/Ammonia Control Product Insert, Bio-Rad Laboratories | | | |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | D. Riedel | Nov. 2000 | Initial Version |
| 2 | L. Lichty | 6/15/2005 |  |
| 3 | L. Lichty | 12/15/06 |  |
| 4 | L. Lichty | April 1, 2011 | New format, package insert revisions. Renumbered from CH 3.09 |
| 5 | L. Lichty | 2/28/13 | Clarify max dilution reporting |
|  | 6 | L. Lichty | 12/19/13 | Revised for new AMM reagent (RXL) |
|  | 7 | L. Lichty | 12/17/13 | Siemens AMM - CLSI Procedure for Vista, Rev B, September 3, 2013 |
|  | 8 | D. Helfinstine/L. Lichty | 8/4/14 | Replaces Ammonia on RxL |
|  | 9 | Erin Bartos | 6/13/2017 | Biennial Review |
|  | 10 | Kelsi Brown | September 6, 2017 | Updated interferences per package insert from Siemens. |
|  | 11 | Erin Bartos | 10/16/2018 | Added rejection of clotted samples |
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