| **Ammonia** |
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| **Purpose** | This procedure provides instructions for performing AMMONIA on ABBOTT INSTRUMENTATION. The Alinity c Ammonia Ultra assay is used for the quantitative enzymatic determination of ammonia in human plasma on the Alinity c analyzer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children’s Minnesota Laboratory.  |
| **Principle** | Ammonia, in the presence of glutamate dehydrogenase (GLDH), combines with α-ketoglutarate and NADH to yield glutamate and NAD+. The decrease in absorbance (NADH to NAD+) at 340 nm is proportional to the ammonia concentration in the examined plasma. The reagent contains lactate dehydrogenase (LDH) in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system.**Methodology:** Glutamate Dehydrogenase |
| **Clinical Significance** | Ammonia, derived from the catabolism of amino acids and from the action of intestinal bacteria on dietary protein, is converted to urea in the liver hepatocytes and so rendered non-toxic. Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances. Elevated ammonia may also be observed in severe liver failure, as may occur in Reye’s Syndrome, viral hepatitis, or cirrhosis. Hyperammonemia occurs with genetic defects of the urea cycle and some other hereditary disorders. Therefore, elevated plasma ammonia may occur in the pediatric population. Elevated ammonia has also been reported due to administration of valproic acid. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code SALIC)****Backup:** Opposite campus. Freeze sample immediately after draw and centrifugation to preserve sample integrity. Samples that give absorbance errors for interferences and cannot be cleared should be sent to Mayo Medical Laboratories using code NH3V. These samples must be frozen within 2 hours of draw; 0.5 mL of EDTA plasma is required. |
| **Sunquest Test Codes** | **NH3** |
| **Specimen** | Sample:  Preferred: EDTA Plasma on iceAlternative: Lithium or Sodium HeparinThe collection tube must be completely filled with blood and immediately placed on ice. The sample must be centrifuged within 15 minutes of draw.**Minimum sample volume:** 0.6 mL, 1.8 mL draw**Stability when separated from cells/gel:** 2 to 8°C 2 Hours (Freeze samples if any delay occurs.) -20°C 3 weeks**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma. Hemolyzed samples should not be used as erythrocytes contain ammonia levels approximately three times that of plasma. If provider requests testing despite hemolysis, send to MML using code NH3V. Samples with visible lipemia or icteremia should be sent to MML if a result cannot be obtained. Ultracentrifigation is contraindicated due to sample stability. Samples not centrifuged within 15 minutes of draw should be recollected.**Preparation:** 1. Samples must be centrifuged within 15 minutes of draw.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of 20 minutes from the time of collection. If there is any delay in testing, freeze specimen. Freeze specimen if sending to MML.
3. Specimens should be free of particulate matter.
4. Transfer or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity c Ammonia Ultra Reagent Kit; includes calibrator | **08P2220** | **Store at:** 2 to 8°C**Unopened: Until expiration Date** **On-board**: 15 days |

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| **Risk and Safety** |

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| Contains methylisothiazolone- may cause allergic skin reaction Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/)  |

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| **Calibration** |

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| Assay Range: | 30 - 998 μmol/L |
| Reference Material: | Alinity c Ammonia Ultra Reagent Kit (Calibrator included in kit) |
| Suggested Calibration Levels: | Calibrator concentration 293.50 μmol/L. The calibrator value is verified using an internal standard obtainedfrom ammonium sulfate puriss. (ultrapure) |
| Calibration Scheme: | Linear data reduction method |
| Calibration Frequency: | 24 hours |
| AMR | AMR is verified twice annually using the Maine Standards GC2 Product # 1200ab by running all applicable levels in triplicate. Assay results are submitted to Maine Standards for compilation and comparison to peers. Results are reviewed and approved by the Technical Specialist. Questionable results are investigated and corrective actions documented. |

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| **Quality Control** | Bio-Rad Liquichek™ Ethanol/Ammonia Levels 1 and 3**Frequency:** Two levels each day of use**Stability:** Stable two years when stored refrigerated between 2 and 8°C. Once opened, and stored tightly capped at 2 to 8°C, this product is stable for 20 days. **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * Minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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| **H** | **I** | **L** |
| **X** | **-** | **X** |

At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interferent: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference”* Hemoglobin: hemolyzed samples should not be used as erythrocytes contain ammonia levels approximately 3 times that of plasma. EDTA plasma samples with visible hemolysis may be sent to Mayo. Green top plasma samples with visible hemolysis should be redrawn.
* Bilirubin: up to 66 mg/dL

For samples with absorbance errors, see limitations. EDTA plasma samples may be sent to Mayo if unable to clear absorbance error. Green top plasma samples should be redrawn if unable to clear absorbance error. * Triglycerides (Intralipid): up to 100mg/dL

Instrument absorbance errors were observed with Intralipid concentrations greater than 100 mg/dL. Do not use samples with visible lipemia. EDTA plasma samples may be sent to Mayo. Green top plasma samples should be redrawn.* Sulfasalazine and its metabolite Sulfapyridine at therapeutic concentrations may lead to falsely low results.
* Temozolomide and its metabolite MTIC may lead to falsely elevated results and its metabolite AIC may lead to falsely low results.
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|  | **0 to 13 days:** 64 - 107 μmol/L**14 to 30 days:** 56 - 92 μmol/L**1 month to <18 years:** <30 - 50 μmol/L**Adult:** <30 - 32 μmol/L |
| **Reference Intervals** |  |
| **Critical Values** | >150 μmol/LResults must be called and documented according to critical values policies. |
| **Limitations** | Sulfasalazine and its metabolite Sulfapyridine at therapeutic concentrations may lead to falsely low results.Temozolomide and its metabolite MTIC may lead to falsely elevated results and its metabolite AIC may lead to falsely low results.**If you receive an absorbance error for Ammonia:**Because this method is colorimetric, a sample that has color other than normal serum color will likely give an absorbance error.Dilute sample using instrument dilution of 1:1.85. If the absorbance error clears AND you get a numerical result greater than 56, you may report the result. If the absorbance error cannot be cleared OR the result is less than 56, attempt manual dilutions of 1:5 and if necessary 1:10. If manual dilutions do not clear the absorbance error, report the sample as “Unable to Determine” UNT. Samples may be sent to Mayo Medical Laboratories for testing if drawn in an EDTA tube. Cancel and reorder NH3V.  |
| **Dilutions** |

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| Max Auto Dilution: | 1: 1.85 |
| Maximum Manual Dilution: | 1:10 |
| Automated Diluent: | Onboard diluent |
| Manual Diluent: | Saline |
| Instrument Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 30 (56 when diluted), do not report the result. Investigate for other possible causes of error or cancel the sample as “Unable to Determine”- UNT. |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming manual dilutions. The system will automatically calculate the concentration of the sample and report the result.If a diluted sample result is less than the lower value of the measuring range of 30 μmol/L**,** do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 30 and 150 without error messages are released
* Results below 30 without error messages are reported as < 30.
* Results > 998 should be diluted using the onboard automated 1:1.85. Release results without error messages following this dilution.
* Results > 1846 following automated dilution are reported as > 1846.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. Alinity Ammonia Ultra Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, March 2018.
2. BioRad Ethanol/Ammonia Control Package Insert, BioRad Laboratories.
3. CALIPER pediatric reference range database. (2020). Retrieved March, 2020, from [https://caliper.research.sickkids.ca/#/](https://caliper.research.sickkids.ca/%23/)
4. [Mayo Medical Laboratories Test Guide](https://www.mayocliniclabs.com/test-catalog/Overview/35130), accessed 11/16/2020.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Stephen Gripentrog, Erin Bartos | 10/28/2020 | New Procedure for Abbott analyzers |
| 2 | Erin Bartos | 11/23/2020 | Added absorbance error information, changed preferred sample type. |
|  |  | Erin Bartos | 12/24/2020 | More clarification on interferences. |