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Kaiser Permanente Medical Center, San Francisco
Northern California Region
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Work Instruction

Title: **AU 680 Ammonia**		**WI Number** SFOWI-1273 **Revision:** 4
Department: Chemistry **Area:** 2425 Geary Blvd SFO Hospital Lab	***Approved & Released Work Instruction***	**Implementation Date:** 04/08/2019
Type of Document: Work Instruction		**Review Period -** 365 **Days**

Intended Use	System reagent for the quantitative determination of in ammonia (NH_3) human plasma on Beckman Coulter AU Clinical Chemistry analyzers.
Principle	The Beckman Coulter AU System Ammonia reagent is a direct enzymatic procedure based on the following reaction sequence. **GLDH** **NH_4 + a-ketoglutarate + NADH---------> Glutamate + NAD + H_2O** The reagent contains LDH in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system. The Beckman Coulter AU Ammonia reagent also incorporates a patented stabilization process which renders the reagent stable in the liquid phase.
Clinical Indication	Ammonia is derived from the catabolism of amino acids and from the action of intestinal bacteria on dietary protein and is converted to urea in the liver hepatocytes and so rendered non-toxic. Under normal circumstances the concentration of ammonia in the circulation remains low, typically less than 50 µmol/L (85 µg/dL). Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances. Elevated levels of ammonia may be either due to inborn errors of metabolism or as secondary to other conditions. Inborn errors of metabolism are the major cause of elevated ammonia in infants and usually the result of urea cycle enzyme deficiencies. Inherited disorders affecting the metabolism of the dibasic amino acids (lysine and ornithine) and those involving the metabolism of organic acids may also produce elevated levels of circulating ammonia. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis, or cirrhosis.

Sample	**Type:** Lithium Heparin Plasma **Volume:** Minimum- 0.5 mL Sample Size (dead space excluded)- 25 uL **Stability:** Refrigerated (2 - 8°C) : 3 hours Frozen (≤-20°C): 24 hours **Unacceptable specimen:** Plasma collected in ammonium heparin **Special Handling** • The collection tube should be completely filled with blood and immediately placed on ice. • Centrifuge the sample as soon as possible for 10 minutes. • If testing cannot be done immediately, separate the plasma and keep at 2 - 8°C until analysis for 24 hours.

Reagent

Preparation:

Beckman Coulter AU System Ammonia Reagent:

Reagent	Ingredient	Concentration	Preparation
R1	a-Ketoglutarate NADH GLDH (Micro-organism) LDH (Micro-organism) Tris buffer	7.5 mmol/L >0.2 mmol/L > 4000 U/L > 30,000 U/L 100 mmol/L	Ready for use
R2	not required	n/a	n/a

Storage and Stability:

	Storage	Expiration Date
Unopened	2 - 8°C	Stable until expiration date on label
Opened	In refrigerated compartment of the analyzer	14 days - Record open date & initials on vial

- Opened bottle expiration date is monitored by the analyzer.
- Do not use the reagent kit or calibrators after the expiration date.

Indications of Deterioration:

Discoloration of the reagent, visible signs of microbial growth, turbidity or precipitation in reagent may indicate degradation and warrant discontinuance of use.

Precautions:

- Reagents contain sodium azide as a preservative. Flush with plenty of water when discarding reagents.

<table>
<tr><td>Calibration</td><td>Frequency:
Perform a single-point calibration (AB) using a water blank and the Beckman Coulter AU System Ammonia Standard (included in the kit).
• At least every 7 days
• Each new reagent lot
• When QC has shifted
• After major preventive maintenance, or replacement of a critical part

Calibrator Name:
Ammonia Standard (included in the kit), 59 umol/L, traceable to NIST Standard Reference Material. Ready to use liquid form.

Preparation, Storage & Stability:
<table>
<tr><th></th><th>Storage</th><th>Expiration Date</th></tr>
<tr><td>Unopened</td><td>2 - 8°C.
Bottles must be capped when not in use.</td><td>Stable until expiration date on label</td></tr>
<tr><td>Opened</td><td>2 - 8°C.
Bottles must be capped when not in use.</td><td>Stable until expiration date on label.
Record open date & initials on bottle.</td></tr>
</table>
</td></tr>
<tr><td>Quality Control</td><td>Frequency:
• Two levels of QC every 24 hours
• Each new reagent bottle (even if same Lot #)
• Each new reagent lot
• After every calibration
• After each shipment of the same Lot #
• After specific maintenance or troubleshooting as detailed in the operators manual or after service/repair performed by Clinical Technology.

Control Name, Preparation, Storage and Stability:
Refer to SFOFCD-0407 AU680 Controls and Calibrators

QC Acceptable Criteria:
Refer to SFOWI-0218 Chem Quality Assurance Plan Section C. Quality Control</td></tr>
<tr><td>Maintenance</td><td>Refer to:
• AU680 Daily Start Up Flow Chart (SFOFCD-0408)
• AU680 Chemistry Analyzer User's Guide-Chapter (8) -Maintenance</td></tr>
<tr><td>Test Procedures</td><td>Refer to AU Operating procedures:
SFOWI-1268 AU680 General Operating Procedures</td></tr>
<tr><td>Calculation</td><td>No calculations are necessary. The results are computed by the instrument.</td></tr>
<tr><td>Analytical Measurement Range (AMR)</td><td>10 - 600 umol/L</td></tr>
</table>

<table>
<tr><td>Reportable Range

(Linear Limits in LIS)-</td><td>10 - 1000 umol/L

Results outside the linear limits are reported as "< 10 umol/L", or "> 1000 umol/L".</td></tr>
<tr><td>Dilution</td><td>
<table>
<tr><td>On-Board Auto Dilution</td><td>Maximum Dilution</td><td>Diluent</td></tr>
<tr><td>x3</td><td>x3</td><td>Deionized water</td></tr>
</table>
NOTE: On-board dilution = Maximum dilution

Auto dilution:
• When results exceed the assay's AMR, an on-board auto-dilution is performed. Results are automatically multiplied by the instrument.

Manual dilution: n/a
</td></tr>
<tr><td>Repeat</td><td>• Follow laboratory repeat policy
• Review instrument printouts for result reasonableness, questionable results are repeated.
• Review instrument printouts for LIH indices and any flags.</td></tr>
<tr><td>Reporting</td><td><u>Confirm ALL flags and indices are properly addressed before reporting any result.</u>

Report Ammonia results in umol/L and in whole number.

Review the instrument printouts for result reasonableness, LIH indices and flags.

If any index shows ABN, visually check the sample appearance to confirm the index ABN is correct. Extreme lipemia, hemolysis, or icterus can show all indices as "ABN".

Severe hemolysis causes false elevations in Ammonia assay. Refer to SFOFCD-0411 "AU680 Comment Codes for Reporting Interference due to Lipemia, Icterus, and Hemolysis"</td></tr>
<tr><td>Reference Intervals</td><td>0 - 150 years: 16 - 53 umol/L</td></tr>
<tr><td>Critical Values</td><td>
<table>
<tr><td>Critical Values</td><td>0 - 18 years: ≥ 200
18 - 150 years: none</td></tr>
</table>
</td></tr>
<tr><td>Early Notification Values</td><td>None</td></tr>
</table>

Interference	• Hemolyzed samples should not be used as erythrocytes contain levels of ammonia axpproimately 3 times that of plasma. • No interference from pyruvate was observed up to a level of 0.75 mmol/L or 0.01 mg/dL. • No interference from ALT was observed up to a level of 2400 U/L. • Avoid contamination from ammonia. Sources of contamination include, but are not restricted to cigarette smoking (patient and collection staff), laboratory atmosphere, laboratory glassware or other reagents on the carousel which contain ammonia. In case of the latter, avoid use of the ammonia containing reagents with OSR61154 to mitigate against atmospheric ammonia transfer. • Bilirubin: No significant interference up to 1.8mg/dL unconjugated bilirubin. • Lipemia: No significant interference up to 100mg/dL triglycerides.
Test Availability	24 hours, routine and stat
Back Up Testing	The second AU 680 Analyzer
References	RWLQCWI-2058 rev.5 AU 680 Ammonia Beckman Coulter Instructions for Use: Ammonia, CLSIOSR61154.02 March 2019 Beckman Coulter Product Announcement: Ammonia Reagent OSR61154, B91129-AB May 2016

Associated Documents:

External Documents

Ammonia PAL, setting sheets and IFU.2016.pdf

Associated Quality System Documents - None

Document Revision History:

Revision: 4	**Date Created:** 08/09/2016 **Date of Last Revision:** 04/08/2019	**Last Approval Date:** 04/08/2019

Document Author: Kevin W LUI/CA/KAIPERM	**Document Manager:** Vaiju Ruikar/CA/KAIPERM

Reason for Change:

Revision:	Sec/Para Changed	Change Made:	Date
1	N/A	Initial Issue of Document. This document was a modification of Regional Lab's AU procedure RWLQCWI-2058 rev. 3.	08/09/2016
2	Edit	Change in Associate Pathologist	10/14/17
3.	Critical and Reference Range. Approver	Added new Critical Value for Age : 0-18 years Ammonia Changed CLIA Director	3/25/2019

Approvals:

Name: Vaiju Ruikar/CA/KAIPERM
Title: Assistant Lab Administrative Directorr

Apr 8, 2019 12:18:01 PM PDT - Approved by: Vaiju Ruikar/CA/KAIPERM

Name: Elizabeth M Hosfield/CA/KAIPERM
Title: Chief of Pathology; CLIA Director

Apr 8, 2019 12:43:01 PM PDT - Approved by: Elizabeth M Hosfield/CA/KAIPERM