ANNUAL REVIEW

Reviewed by: | Date | Reviewed by: | Date
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PRINCIPLE

INTENDED USE

A1M reagent, when used in conjunction with IMMAGE® Immunochemistry Systems and Urine Protein Calibrator, is intended for the quantitative determination of alpha-1-microglobulin (A1M) in human urine by rate nephelometry.

CLINICAL SIGNIFICANCE

Measurement of alpha-1-microglobulin in urine aids in the diagnosis of kidney tubular damage, which can occur in the course of nephritis, advanced diabetic nephropathy, after heavy metal exposure, and after dosage with nephrotoxic medications.\(^1\),\(^2\),\(^3\),\(^4\),\(^5\),\(^6\) Elevated levels of alpha-1-microglobulin in patients with urinary tract infections indicate kidney involvement.\(^4\)

METHODOLOGY

The A1M test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

CHEMICAL REACTION SCHEME

\[
\text{Alpha-1-microglobulin(sample) + Antibody} \rightarrow [\text{Alpha-1-microglobulin(sample)-Antibody (aggregates)}]
\]

SPECIMEN

TYPE OF SPECIMEN

Urine is the only sample type recommended.

There are no special dietary requirements prior to sample collection. The type of sample collection depends on how results are to be reported.\(^1\) For direct quantitative values, any urine sample is acceptable, with 24-hour collections preferable.\(^7\),\(^8\) Centrifuge urine samples at 3,000 \(x\) g for 10 minutes prior to analysis to remove any cells or other debris.
SPECIMEN STORAGE AND STABILITY

Urine samples should be collected without a preservative and allowed to come to room temperature prior to use. Samples may be stored at +2°C to +8°C for up to 7 days. Frozen samples are not recommended.

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

For sample volumes refer to the Sampling Template.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:
### KIT COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1M Cartridge</td>
<td>1</td>
</tr>
<tr>
<td>Antibody</td>
<td></td>
</tr>
<tr>
<td>Antigen Excess Solution (AGXS)</td>
<td></td>
</tr>
<tr>
<td>Evaporation Caps</td>
<td>2</td>
</tr>
<tr>
<td>A1M Reagent Bar Code Card</td>
<td>1</td>
</tr>
</tbody>
</table>

### INITIAL VOLUMES OF SAMPLE AND REAGENTS IN THE CUVETTE

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>21 µL</td>
</tr>
<tr>
<td>Total Reagent Volume</td>
<td>321 µL</td>
</tr>
<tr>
<td>Antibody</td>
<td>21 µL</td>
</tr>
<tr>
<td>Buffer 1</td>
<td>300 µL</td>
</tr>
</tbody>
</table>

### REACTIVE INGREDIENTS

<table>
<thead>
<tr>
<th>Reagent Cartridge Constituents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1M Antibody (processed goat sera)</td>
<td>3.9 mL</td>
</tr>
<tr>
<td>A1M Antigen Excess Solution</td>
<td>3.9 mL</td>
</tr>
<tr>
<td>Sodium Azide (used as a preservative)</td>
<td>&lt; 0.1% (w/w)</td>
</tr>
</tbody>
</table>

Also non-reactive chemicals necessary for optimal system performance.

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⚠️ **CAUTION**

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

⚠️ **CAUTION**

Although not composed of substances of human origin, this product may come in contact with human serum during processing. This material and all patient samples should be handled as though capable of transmitting infectious disease. The United States Food and Drug Administration recommends such samples be handled as specified in the Centers for Disease Control's Biosafety Level 2 guidelines.

### MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

- IMMAGE Immunochemistry Systems Wash Solution
- IMMAGE Immunochemistry Systems Buffer 1
- IMMAGE Immunochemistry Systems Diluent 1
- Urine Protein Calibrator
- Centrifuge capable of 3,000 x g
- At least two levels of control material
REAGENT PREPARATION

1. Invert cartridge gently before removing screw caps.
2. Remove screw caps from reagent cartridges. Check each cartridge for bubbles and remove any bubbles present.
3. Place evaporation caps on both reagent cartridge compartments before loading the cartridge on the instrument. See Appendices for evaporation cap directions.
4. Reagent cartridges should be stored upright and can be removed from the refrigerator and used immediately.
5. Mix all buffers and diluents thoroughly by inversion. Remove screw cap from container. Check each container for bubbles and remove any bubbles present. Place evaporation cap on container before loading the container on the instrument. See Appendices for evaporation cap directions.

ACCEPTABLE REAGENT PERFORMANCE

Acceptability of a reagent is determined from the successful performance of quality control testing, as defined in the QUALITY CONTROL section of this chemistry information sheet.

REAGENT STORAGE AND STABILITY

Storage conditions other than those recommended may cause erroneous results.

Reagent Cartridges

1. Return all reagent cartridges to the refrigerator (+2°C to +8°C) upon completion of the daily workload.
2. The A1M reagents are stable for 30 days with the evaporation caps in place. Alternatively, reagent life can be maximized by replacing evaporation caps with screw caps and storing at +2°C to +8°C upon completion of the daily workload.
3. The A1M reagents are stable until the expiration date on the label if the reagents are stored at +2°C to +8°C with the screw caps in place.

Diluent 1 and Buffer 1

1. Diluent 1 and Buffer 1 are stable on the system for 30 days with the evaporation caps in place.
2. Diluent 1 and Buffer 1 are stable until the expiration date on the label if they are stored at room temperature with the screw caps in place.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

Urine Protein Calibrator

CALIBRATOR PREPARATION

No preparation is required.
CALIBRATOR STORAGE AND STABILITY

The calibrator is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

Calibrator storage location:

⚠️ CAUTION

Urine is not known to transmit infectious disease such as Hepatitis or HIV. However, because this product contains material of human origin, it should be handled as though capable of transmitting infectious diseases. The United States Food and Drug Administration recommends such samples be handled as specified in the Centers for Disease Control's Biosafety Level 2 guidelines.9

IMMAGE IMMUNOCHEMISTRY SYSTEM CALIBRATION INFORMATION

1. The IMMAGE® Immunochemistry Systems calibration is reagent lot specific.

2. The A1M reagent lot should be recalibrated when changing Buffer 1 lot or following specific part replacements or maintenance procedures as defined in the IMMAGE Operations Manual.

3. The IMMAGE Immunochemistry System is designed for minimum calibration. Calibrations retained in system memory should be monitored by the performance of quality control procedures on each day of testing.

4. Calibration for A1M is stable for 30 days.

5. The system will automatically perform a verification check during calibration and produce a calibration report. The system will alert the operator of a failed calibration. An explanation of any accompanying error message can be found in the TROUBLESHOOTING Section of the IMMAGE® Immunochemistry Systems Operations Manual.

6. Calibration verification information can be found in the CALIBRATION VERIFICATION section of the IMMAGE® Immunochemistry Systems Chemistry Reference Manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

It is recommended that at least two levels of control material, normal and abnormal, be analyzed daily. Refer to the CALIBRATORS AND CONTROLS section of the IMMAGE® Immunochemistry Systems Chemistry Reference Manual, for a list of Beckman Coulter controls. Controls should also be run with each new calibration, with a new lot of reagent or buffer, and after specific maintenance or troubleshooting as detailed in the IMMAGE® Immunochemistry Systems Operations Manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on work load and work flow.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.
Table 1.0 Quality Control Material

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

TESTING PROCEDURE(S)

1. After setup, load reagents onto the system as directed in the IMMAGE Operations Manual.
2. Select chemistries to be calibrated, if necessary. Load bar coded calibrators, controls, and samples or program and load non-bar coded controls and samples for analysis as directed in the IMMAGE Operations Manual.
3. Follow the protocols for system operation as directed in the IMMAGE Operations Manual.

CALCULATIONS

The IMMAGE Immunochemistry System will automatically calculate results.

REPORTING RESULTS

REFERENCE INTERVALS

The reference range values for human alpha-1-microglobulin in random urine were established using the IMMAGE test, for a population of 123 apparently healthy male and female adults, Ames Multistix protein negative, from California. The reference range values for human alpha 1-microglobulin in 24 hour urine collections were established using an Array® 360 System, for a population of 25 apparently healthy male and female adults from California.

Table 2.0 Reference intervals*

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter Urine (random)</td>
<td>&lt; 1.25 mg/dL (in 99% of the population tested)</td>
</tr>
<tr>
<td>Beckman Coulter Urine (24 hour)</td>
<td>1.28 mg/dL (in 95% of the population tested)</td>
</tr>
</tbody>
</table>

a Each laboratory should establish its own reference interval(s) based on its patient population.

Table 2.0 Reference intervals (for Laboratory specific)

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>REFERENCE INTERVALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Refer to References (10,11,12,13) for guidelines on establishing laboratory-specific reference intervals.
Additional reporting information as designated by this laboratory:

UNITS AND CONVERSION FACTOR

Results for the A1M test are reported in default units of mg/dL. Metric conversion within the same unit category will occur automatically if a new unit is selected. A conversion factor must be entered when selecting a unit category different from the default.

Refer to the System Setup section of the IMMAGE Operations Manual for more detailed information on units and conversion factors.

PROCEDURAL NOTES

LIMITATIONS

Urine samples that have been frozen or contaminated with blood are not recommended.

INTERFERENCES

Dust particles or other particulate matter (i.e. debris and bacteria) in the reaction solution may result in extraneous light-scattering signals, resulting in variable sample analysis.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The A1M test is designed to detect concentrations of this analyte using undiluted (neat) urine samples.

Table 3.0 Analytical Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>BECKMAN COULTER ANALYTICAL RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Initial: 0.4 – 8.0 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Extended: 0.4 – 1,728 mg/dL</td>
</tr>
</tbody>
</table>

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 4.0 Reportable Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>LABORATORY REPORTABLE RANGE</th>
</tr>
</thead>
</table>

Refer to the IMMAGE® Immunochemistry Systems Chemistry Reference Manual section on CALIBRATION VERIFICATION, for more details on laboratory reportable range.

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for A1M determination is 0.4 mg/dL.
EQUIVALENCY

Equivalency was assessed by Deming regression analysis of samples to an accepted clinical method. Values obtained for human alpha-1-microglobulin using the IMMAGE® Immunochemistry Systems A1M test were compared to the values obtained using an Array® 360 System. Both normal and abnormal alpha-1-microglobulin urine samples were included in the analysis.

Table 5.0 Equivalency Values

<table>
<thead>
<tr>
<th>TYPE OF MEASUREMENT</th>
<th>SAMPLE TYPE</th>
<th>SD (mg/dL)</th>
<th>% CV</th>
<th>CHANGEOVER VALUE (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Urine</td>
<td>0.08</td>
<td>5.0</td>
<td>1.60</td>
</tr>
<tr>
<td>Total</td>
<td>Urine</td>
<td>0.12</td>
<td>6.0</td>
<td>2.00</td>
</tr>
</tbody>
</table>

The equivalency values were determined using patient samples ranging from 0.41 to 7.21 mg/dL. Refer to References (14,15) at the end of this chemistry information sheet for guidelines on performing equivalency testing.

PRECISION

A properly operating IMMAGE® Immunochemistry Systems should exhibit imprecision values less than or equal to the maximum performance limits listed below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 6.0 Maximum Performance Limits

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE</th>
<th>SD (mg/dL)</th>
<th>% CV</th>
<th>CHANGEOVER VALUE (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Urine</td>
<td>0.08</td>
<td>5.0</td>
<td>1.60</td>
</tr>
<tr>
<td>Total</td>
<td>Urine</td>
<td>0.12</td>
<td>6.0</td>
<td>2.00</td>
</tr>
</tbody>
</table>

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the IMMAGE® Immunochemistry Systems evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below. Each laboratory should characterize their own instrument performance for comparison purposes.

Table 7.0 Typical Imprecision Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE</th>
<th>Data Pointsa</th>
<th>Test Mean Value (mg/dL)</th>
<th>SD (mg/dL)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Urine Level 1</td>
<td>80</td>
<td>0.77</td>
<td>0.021</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Urine Level 2</td>
<td>80</td>
<td>3.05</td>
<td>0.056</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Urine Level 3</td>
<td>80</td>
<td>6.59</td>
<td>0.161</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Table 7.0 Typical Imprecision Values, Continued

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE</th>
<th>Data Points*</th>
<th>Test Mean Value (mg/dL)</th>
<th>SD (mg/dL)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Urine Level 1</td>
<td>80</td>
<td>0.77</td>
<td>0.031</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Urine Level 2</td>
<td>80</td>
<td>3.05</td>
<td>0.061</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Urine Level 3</td>
<td>80</td>
<td>6.59</td>
<td>0.194</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* The point estimate is based on the data from 1 system, run for 20 days, 2 runs per day, 2 observations per run on an instrument operated and maintained according to the manufacturer’s instructions.

Refer to References (14,16) for guidelines on performing precision testing.

NOTICE

These degrees of precision were obtained in typical testing procedures and are not intended to represent performance specifications for this test procedure.

ADDITIONAL INFORMATION

For more information, refer to the IMMAGE Immunochemistry Systems Operations Manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

FOOTNOTES

* Multistix is a registered trademark of Ames.
REFERENCES


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Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068)

Beckman Coulter, Inc., 250 South Kraemer Blvd., Brea, CA 92821