For In Vitro Diagnostic Use

ANNUAL REVIEW

<table>
<thead>
<tr>
<th>Reviewed by:</th>
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PRINCIPLE

INTENDED USE

LPAX reagent, when used in conjunction with IMMAGE® Immunochemistry Systems and Lipoprotein(a) Calibrator, is intended for the quantitative determination of lipoprotein(a) (LPAX) in human serum or plasma by rate nephelometry.\(^1,2\)

CLINICAL SIGNIFICANCE

The measurement of lipoprotein(a), in conjunction with other lipoprotein tests, is of diagnostic significance when assessing atherosclerotic cardiovascular disease in specific populations.

METHODOLOGY

The LPAX 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

\[
\text{Lipoprotein(a)(sample) + Particle bound anti-Lp(a) (antibody) \rightarrow [Lipoprotein(a)(sample)-Antibody Complex]}
\]

SPECIMEN

TYPE OF SPECIMEN

Freshly drawn serum from a fasting individual is preferred. Plasma samples (EDTA, Lithium Heparin, and Sodium Heparin) can be used.

Serum or plasma samples should be collected in the manner routinely used for any clinical laboratory test.\(^3\) Anticoagulants tested are listed in the PROCEDURAL NOTES section of this chemistry information sheet.

For analysis of lipemic samples, refer to the listing of interferences in the PROCEDURAL NOTES section of this chemistry information sheet.
SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum be physically separated from contact with cells within two hours from the time of collection. If samples are not assayed within 8 hours, samples should be stored at +2°C to +8°C. If samples are not assayed within 48 hours, samples should be stored frozen at -70°C or below. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed. Limited testing indicates that samples stored for 5 months at -70°C were stable within the precision of the assay.

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:

<table>
<thead>
<tr>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPAX Cartridge</td>
<td>1</td>
</tr>
<tr>
<td>Antibody</td>
<td></td>
</tr>
<tr>
<td>Evaporation Caps</td>
<td>2</td>
</tr>
<tr>
<td>LPAX Reagent Bar Code Card</td>
<td>1</td>
</tr>
</tbody>
</table>

INITIAL VOLUMES OF SAMPLE AND REAGENTS IN THE CUVETTE

- Sample Volume: 0.56 µL
- Total Reagent Volume: 340.44 µL
  - Antibody: 21 µL
  - Buffer 1: 300 µL
  - Diluent 2: 19.44 µL

REACTIVE INGREDIENTS

<table>
<thead>
<tr>
<th>REAGENT CARTRIDGE CONSTITUENTS</th>
<th>VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPAX Antibody (particle bound polyclonal anti-lipoprotein(a) antibody; rabbit)</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 bovine serum albumin and non-reactive chemicals necessary for optimal system performance.

⚠️ 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 2
- Lipoprotein(a) Calibrator
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 LPAX reagent is 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 LPAX reagent is stable until the expiration date on the label if stored at +2°C to +8°C with the screw caps in place.

Diluent 2 and Buffer 1

1. Diluent 2 and Buffer 1 are stable on the system for 30 days with the evaporation caps in place.
2. Diluent 2 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

Lipoprotein(a) Calibrator

CALIBRATOR PREPARATION

Allow LPA Calibrator to come to room temperature prior to use. Reconstitute LPA Calibrator with 1.0 mL of distilled water, then let stand at room temperature for 2 hours. Mix by inversion before use.
CALIBRATOR STORAGE AND STABILITY

Lipoprotein(a) 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. After reconstitution, Lipoprotein(a) Calibrator is stable for 1 month at +2°C to +8°C or for 3 months at -15°C to -20°C, unless the expiration date is exceeded.

Calibrator storage location:

⚠️ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.5

IMMAGE IMMUNOCHEMISTRY SYSTEM CALIBRATION INFORMATION

1. The IMMAGE® Immunochemistry Systems calibration is reagent lot specific.
2. The LPAX reagent lot should be recalibrated when changing Buffer 1 or Diluent 2 lots, or with 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 LPAX 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>
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<tbody>
<tr>
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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 LPAX reference interval values for human serum lipoprotein(a) were established using the IMMAGE and the Array® 360 Immunochemistry Systems, for a population of African-American males (ages 20-63) and females (ages 20-81), and Caucasian males (ages 25-81) and females (ages 24-85) from the U.S.A., having a normal lipid profile as defined by the National Institute of Health (NIH), National Cholesterol Education Program (NCEP), with total cholesterol levels of ≤239 mg/dL.6,7

Table 2.0 Reference intervals

<table>
<thead>
<tr>
<th>LIPOPROTEIN(a) CONCENTRATION, (mg/dL)⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>African-American</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
</tbody>
</table>

a Each laboratory should establish its own reference interval(s) based on its patient population.6,9
b Expressed as anti-log transformed concentration defined as the 25th to the 75th percentile.
Refer to References (10,11,12) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

### UNITS AND CONVERSION FACTOR

Results for the LPAX 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

#### ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with 47 paired serum and plasma samples. Values of serum (X) ranging from 3.28 mg/dL to 108 mg/dL were compared to values for plasma (Y) yielding the following results:

**Table 3.0 Anticoagulant Test Results**

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>LEVEL OF ANTICOAGULANT TESTED</th>
<th>DEMING REGRESSION ANALYSIS (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Heparin</td>
<td>14 Units/mL</td>
<td>( Y = 0.985X - 0.508; \ r = 0.999 )</td>
</tr>
<tr>
<td>Sodium Heparin</td>
<td>14 Units/mL</td>
<td>( Y = 0.995X - 0.637; \ r = 0.999 )</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.5 mg/mL</td>
<td>( Y = 0.949X + 0.074; \ r = 0.999 )</td>
</tr>
</tbody>
</table>

#### INTERFERENCES

1. The following substances were tested in serum for interference with this methodology at the initial dilution:

**Table 4.0 Interferences**

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>Lp(a) CONCENTRATION</th>
<th>OBSERVED EFFECT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen</td>
<td>Human</td>
<td>100 mg/dL</td>
<td>11 – 89 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Human</td>
<td>100 – 500 mg/dL</td>
<td>6 – 82 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Porcine</td>
<td>5 – 30 mg/dL</td>
<td>6 – 85 mg/dL</td>
<td>NSI</td>
</tr>
</tbody>
</table>
Table 4.0 Interferences, Continued

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>Lp(a) CONCENTRATION</th>
<th>OBSERVED EFFECT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipidb</td>
<td>Intralipidc</td>
<td>125 – 500 mg/dL</td>
<td>5 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 – 1000 mg/dL</td>
<td>5 mg/dL</td>
<td>-1.0 – -2.0 mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125 – 750 mg/dL</td>
<td>38 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 mg/dL</td>
<td>38 mg/dL</td>
<td>-12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125 – 1000 mg/dL</td>
<td>82 mg/dL</td>
<td>NSI</td>
</tr>
</tbody>
</table>

a  Plus (+) or minus (-) signs in this column signify positive or negative interference. NSI: No significant interference. For Lp(a) values ≤8.0 mg/dL, interference is ≤ ±0.8 mg/dL; >8.0 mg/dL, interference is ≤±10%.
b  Quantitation of Lp(a) by nephelometry may not be possible in lipemic specimens, or may produce inaccurate results due to the extreme light scattering properties of the sample.
c  Intralipid is a registered trademark of KabV/trim, Inc., Clayton, NC 27250.

2. Nonspecific interference can occur between less dilute samples and polymer-enhanced buffer when dilutions less than 1:36 are assayed.

3. 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 LPAX test is designed to detect concentrations of this analyte using an initial 1:36 sample dilution.

Table 5.0 Analytical Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>BECKMAN COULTER ANALYTICAL RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/Plasma</td>
<td>Initial: 2.00 – 128 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Extended: 2.00 – 640 mg/dL</td>
</tr>
</tbody>
</table>

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>LABORATORY REPORTABLE RANGE</th>
</tr>
</thead>
<tbody>
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</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 LPAX determination is 2.00 mg/dL.

METHODS COMPARISON

Methods comparison was assessed by Deming regression analysis of samples to an accepted clinical method. Values obtained using the IMMAGE LPAX test were compared to the values obtained using the APO-Tek Lp(a)™ ELISA method. Serum samples from normal, and atherosclerotic, African-American and Caucasian patients were included.
Table 7.0 Methods Comparison Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</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>Serum/Plasma</td>
<td>0.4</td>
<td>5.0</td>
<td>8.00</td>
</tr>
<tr>
<td>Total</td>
<td>Serum/Plasma</td>
<td>0.6</td>
<td>6.5</td>
<td>9.23</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 8.0 Maximum Performance Limits

The methods comparison values were determined using patient samples ranging from 2.1 to 364.0 mg/dL. Refer to References (13,14) at the end of this chemistry information sheet for guidelines on performing methods comparison testing.

Deming regression analysis of the methods comparison study by race and sex produced the following results:

African-American females: \( Y = 0.789 + 5.85; r = 0.928; SE = 0.037; n = 75 \)
African-American males: \( Y = 0.868 + 0.874; r = 0.937; SE = 0.041; n = 64 \)
Caucasian females: \( Y = 0.812 - 0.901; r = 0.943; SE = 0.027; n = 118 \)
Caucasian males: \( Y = 0.761 + 1.33; r = 0.934; SE = 0.025; n = 143 \)

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 9.0 Typical Imprecision Values

<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>Within-run</td>
<td>Serum Level 1</td>
<td>80</td>
<td>6.57</td>
<td>0.207</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Serum Level 2</td>
<td>80</td>
<td>40.0</td>
<td>1.10</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Serum Level 3</td>
<td>80</td>
<td>88.8</td>
<td>2.67</td>
<td>3.0</td>
</tr>
</tbody>
</table>
Table 9.0 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>Serum Level 1</td>
<td>80</td>
<td>6.57</td>
<td>0.232</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Serum Level 2</td>
<td>80</td>
<td>40.0</td>
<td>1.24</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Serum Level 3</td>
<td>80</td>
<td>88.8</td>
<td>3.19</td>
<td>3.6</td>
</tr>
</tbody>
</table>

a 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 (13,15) 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.*

SUGGESTED READING


SHIPPING DAMAGE

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


Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068)
Beckman Coulter, Inc., 250 South Kraemer Blvd., Brea, CA 92821