Establishing new positive ranges for anti-phospholipid antibody tests based on the local population

Dennis Sosnovske
D G. Grenache
Tricore
P Lowrey
D Rospopo
Tricore
K Riley
Tricore

See next page for additional authors

Follow this and additional works at: https://digitalrepository.unm.edu/hsc_qips

Recommended Citation
Sosnovske, Dennis; D G. Grenache; P Lowrey; D Rospopo; K Riley; R A. Marlar; and M A. Rollins-Raval. "Establishing new positive ranges for anti-phospholipid antibody tests based on the local population." (2020). https://digitalrepository.unm.edu/hsc_qips/15

This Presentation is brought to you for free and open access by the Health Sciences Center Events at UNM Digital Repository. It has been accepted for inclusion in Quality Improvement/Patient Safety Symposium by an authorized administrator of UNM Digital Repository. For more information, please contact amywinter@unm.edu, lsloane@salud.unm.edu, sarahrk@unm.edu.
Authors
Dennis Sosnovske, D G. Grenache, P Lowrey, D Rospopo, K Riley, R A. Marlar, and M A. Rollins-Raval

This presentation is available at UNM Digital Repository: https://digitalrepository.unm.edu/hsc_qips/15
Establishing new positive ranges for anti-phospholipid antibody tests based on the local population

Dennis Sosnovske, MD, MS Ed
Pathology Resident, PGY-3
University of New Mexico
Department of Pathology
Disclosures

• Dr. Sosnovske has no conflicts of interest to disclose
Learning Objective

• Understand how the initial verification of a lab instrument can affect the results of a test, and how it can impact a patient.
Brief outline

• Part 1: Instrument validation and verification.
• Part 2: Local population study, and establishing new cutoff values.
• Part 3: Look back to determine how new cutoff values would have affected 1 year of test results.
Introduction to APAS

• Antiphospholipid antibody syndrome (APAS) is an autoimmune disorder that is caused by a person making antibodies to phospholipids that are found on their own cell membranes.

• Results in an abnormally long aPTT

• Clinical consequences of this can range from no symptoms to spontaneous venous thromboembolism (VTE), or spontaneous pregnancy loss.
Consequences of APAS

- Many people with symptomatic APAS need to have anticoagulation and/or antiplatelet therapy for life.
  - Increase risk of bleeding
Part 1: The Setup

• TriCore uses the BioPlex platform (Bio-Rad Laboratories, Hercules, CA) for testing antiphospholipid antibody syndrome (APAS), which is an FDA approved test.

• ELISA test for:
  – anti-cardiolipin (aCL) IgA, IgG, and IgM
  – anti-beta2-glycoprotein 1 (aB2GP1) IgA, IgG, and IgM

• At the time of instillation, Bio-Rad gave a positive cut off off value of 20 U/mL.

• Bio-Rad recommended that cut off values be established based on a $99^{th}$ percentile of the local population.
Where did the 20 U/mL cutoff value come from?

- FDA validation study (from the machine documentation)
- Set-up verification (available samples)
- It is unclear why the manufacturer recommended a cutoff value of 20 U/mL

Table 1: APAS 99th percentiles from validation and verification studies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BioRad validation (n=300)</th>
<th>Initial verification (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2-glycoprotein-1 IgA (U/mL)</td>
<td>12.1</td>
<td>42</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgG (U/mL)</td>
<td>6</td>
<td>23.8</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgM (U/mL)</td>
<td>19.4</td>
<td>22.9</td>
</tr>
<tr>
<td>Anti-cardiolipin IgA (APL-U/mL)</td>
<td>14.5</td>
<td>45.5</td>
</tr>
<tr>
<td>Anti-cardiolipin IgG (GPL-U/mL)</td>
<td>8.5</td>
<td>27.6</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM (MPL-U/mL)</td>
<td>27.9</td>
<td>19.6</td>
</tr>
</tbody>
</table>
It becomes much less clear.

- There are no international standards in place for the detection APAS antibodies.
- Different manufacturers use different monoclonal antibodies for detection.
- Leads to a high degree of variability between commercially available tests for APAS.
- Increases the importance of establishing a local population norm for the tests.
Part 2: The study

• Introduction:
• We wanted to establish a local population cutoff for the APAS tests.
• To do this we proposed collecting 120 samples from a normal local population.
Methods

• Whole blood samples in sodium citrate were collected from 120 healthy donors.
• Stored at -70 degrees C for up to 12 months.
• Concentrations of aCL and aB2GP1 were determined using the BioPlex 2200 System.

• The 99th percentile for each part of the assay was determined, and implemented as new cut off value (starting 1/15/2020)
Table. 99th percentile determinations from validation study, verification study and local population study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BioRad validation (n=300)</th>
<th>Initial verification (n=37)</th>
<th>Local population (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2-glycoprotein-1 IgA (U/mL)</td>
<td>12.1</td>
<td>42</td>
<td>10.6</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgG (U/mL)</td>
<td>6</td>
<td>23.8</td>
<td>6.3</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgM (U/mL)</td>
<td>19.4</td>
<td>22.9</td>
<td>20.1</td>
</tr>
<tr>
<td>Anti-cardiolipin IgA (APL-U/mL)</td>
<td>14.5</td>
<td>45.5</td>
<td>10</td>
</tr>
<tr>
<td>Anti-cardiolipin IgG GPL-U/mL</td>
<td>8.5</td>
<td>27.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM MPL-U/mL</td>
<td>27.9</td>
<td>19.6</td>
<td>25.9</td>
</tr>
</tbody>
</table>
Part 3: look back for impact

- Newly derived cutoffs were applied to 1,118 aCL and 1,140 aB2GP1 results retrieved from the TriCore data warehouse over a 12-month period (1/1/2018 to 12/31/2018).
## Results

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BioRad validation (n=300)</th>
<th>Initial verification (n=37)</th>
<th>Local population (n=120)</th>
<th>Number positive (% of total) with manufacturer’s proposed cutoff</th>
<th>Number positive (% of total) with NM 99th percentile cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2-glycoprotein-1 IgA (U/mL)</td>
<td>12.1</td>
<td>42</td>
<td>10.6</td>
<td>11/1,140 (1.0)</td>
<td>26/1,140 (2.3)</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgG (U/MI)</td>
<td>6</td>
<td>23.8</td>
<td>6.3</td>
<td>12/1,140 (1.1)</td>
<td>35/1,140 (3.1)</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgM (U/mL)</td>
<td>19.4</td>
<td>22.9</td>
<td>20.1</td>
<td>11/1,140 (1.0)</td>
<td>11/1,140 (1.0)</td>
</tr>
<tr>
<td>Anti-cardiolipin IgA (APL-U/mL)</td>
<td>14.5</td>
<td>45.5</td>
<td>10</td>
<td>14/1,118 (1.3)</td>
<td>25/1,118 (2.2)</td>
</tr>
<tr>
<td>Anti-cardiolipin IgG GPL-U/mL</td>
<td>8.5</td>
<td>27.6</td>
<td>9.6</td>
<td>12/1,118 (1.1)</td>
<td>23/1,118 (2.1)</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM MPL-U/mL</td>
<td>27.9</td>
<td>19.6</td>
<td>25.9</td>
<td>15/1,118 (1.3)</td>
<td>8/1,118 (0.7)</td>
</tr>
</tbody>
</table>
Results

• Based on our population’s 99th percentile cut-off values, 27 previously negative individuals would now be labeled positive, whereas only 3 previously positive individuals would now be labeled as negative; the majority of patient results (97.4% of tests) did not change.

• Note: this is a battery of tests, and a result is dependent on the overall pattern of testing, as well as a second test at least twelve weeks apart.
Conclusion

• Given guideline recommendations that a local population be used to establish cut-off values, TriCore Reference Laboratories have changed the cut-off values to the 99th percentile of the local population.

• The 99th percentile results from this study were similar to those established by Bio-Rad Laboratories during their validation.

• It is unclear why a uniform value of greater than or equal to 20 units was applied as the FDA-cleared cut-off.
Further work

• It would be interesting to send the samples to a lab that assays the APAS with a different method, and compare the results.
• Request IRB approval for evaluate the clinical significance for the changes in reference ranges.
Thanks to:

- TriCore special coagulation group
- Dr. Grenache
- Dr. Marlar
- Dr. Rollins-Raval