Lot-to-Lot Inconsistency of Anticardiolipin Reagents

To the Editor:

The diagnostic criteria for antiphospholipid syndrome include the presence of one or more typical clinical features plus one or more laboratory findings (1). The latter include positivity (on two or more occasions, ≥6 weeks apart) of either lupus anticoagulant or anticardiolipin antibody (ACA).

We report inconsistencies among lots of anticardiolipin reagents from one supplier and suggest that the differences are related to changes in calibration materials that are also used by other suppliers of ACA reagents.

Several, perhaps most, ACA ELISAs are calibrated with Harris “standards” (Louisville APL Diagnostics, Inc.) or secondary calibrators that are traceable to them. Recently, there has been a change in the latest generation of calibration materials, the LAPL-GM-200 calibrators for IgG and IgM ACA. When the latest LAPL-GM-200 calibrators were produced, the manufacturer attempted to make these new calibrators agree with their three previous versions, LAPL-GM-100 (distributed 1997–2001), LAPL-GM-001 (1990–1997), and the originals (made before 1990).

We have been using ACA assays (QUANTA Lite™ Anticardiolipin IgG/IgM ELISA HRP Kit; INOVA Diagnostics) that use the Harris calibrators. In October 2001, we received a new shipment of both ACA IgM (lot no. 170264) and IgG (lot no. 170276) reagent sets, both based on the new LAPL-GM-200 calibrators. During routine checking of patient samples with the old and new reagent sets, we found a large negative proportional bias in the IgM results \[y = 0.58x + 3 \text{ MPL}\] (MPL is the conventional IgM ACA unit nomenclature; 1 MPL is the cardiolipin binding activity of 1 mg/L of an affinity-purified IgM); \(r = 0.992\); Fig. 1A] and a large positive proportional bias in the IgG results \[y = 1.34x + 5 \text{ GPL}\]; \(r = 0.997\); data not shown). Concerns were relayed to INOVA. Subsequently (January 2002), we received reformulated reagent sets based on the GM-200 calibrators; these were prepared to better align the assays with results obtained with their previous reagents, which were calibrated with LAPL-GM-100 materials. The reformulated assay produced better agreement for the IgG when compared with assays calibrated with the LAPL-GM-100 mater-

![Fig. 1. Comparison of three lots of QUANTA Lite ACA IgM ELISA reagents.](https://academic.oup.com/clinchem/article/48/9/1625/5642270)
rals (data not shown). However, the prior negative bias of the IgM was overcompensated for; when the revised reagent set (which contained INOVA’s in-house secondary calibrators traceable to the GM-200 material) was compared with the previous lot (no. 170264), we found the following bias: $y = 2.45x - 16$ MPL ($r = 0.95$; Fig. 1B). Comparison of the revised lot and our last lot (170105) that was based on the prior GM-100 standards (170355) showed a slope $>1.0$ and a negative intercept (Fig. 1C).

A semiquantitative assay with categorical limits (i.e., negative, low, medium, high positive) requires consistency across reagent lots. The INOVA product insert suggests that results $<12.5$ MPL be classified as negative, results $\geq 12.5$ to $20$ MPL be classified as indeterminate, and results $>20$ MPL be reported as positive (with $20$–$80$ MPL as low/medium and $>80$ MPL as high). For the last 397 patients that we tested with LAPL-GM-100 reagent sets, results for $31\%$ of the patients were $>20$ MPL. Extrapolating from panels A and C in Fig. 1 would suggest that this percentage would have been $16\%$ with lot no. 170264 and $27\%$ with lot no. 170355.

We appreciate that INOVA has listened to our concerns, but we feel it is important to alert the users of these products to the potential need to readjust their cutoff values when systematic changes occur with new lots of reagents.

### Table 1. Results obtained for sample ACL-04 in the College of American Pathologists survey.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>No. of laboratories</th>
<th>CV, %</th>
<th>Median, units</th>
<th>Low value, units</th>
<th>High value, units</th>
</tr>
</thead>
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<tr>
<td>INOVA</td>
<td>79</td>
<td>12.1</td>
<td>44</td>
<td>26</td>
<td>75</td>
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<tr>
<td>Corgenix</td>
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<td>21.6</td>
<td>16</td>
<td>9</td>
<td>25</td>
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<td>Sigma</td>
<td>11</td>
<td>30.9</td>
<td>43</td>
<td>31</td>
<td>78</td>
</tr>
<tr>
<td>Binding Site</td>
<td>11</td>
<td>25.7</td>
<td>48</td>
<td>33</td>
<td>68</td>
</tr>
</tbody>
</table>

Representatives of INOVA Diagnostics respond to the letter by Drs. Hoefner and Yeo:

To the Editor:

Anticardiolipin antibody (ACA) tests are among the most difficult of all ELISAs to standardize. There are the well-known difficulties of adhering the phospholipid to a plastic microwell plate. In addition, the antigen solid phase is complex, consisting of both the phospholipid plus a necessary cofactor, known as $\beta_2$-glycoprotein ($\beta_2$-GPI), and the blocking agent. Then there is the added problem of having to calibrate each reagent set to a reference preparation that consists of pooled human sera. As mentioned by Drs. Hoefner and Yeo, there have been four different variations of these standards over the years, and despite the best efforts of the producers of these standards, some drift can occur at different parts of the assay range.

It is for these reasons that experts in the ACA field, including those responsible for producing the standards in question, recommend that results be reported in a semiquantitative manner. It has been further recommended that only moderate or high concentrations of IgG and IgM ACA be considered diagnostically important and that two positive results obtained 6 or more weeks apart are necessary.

Shown in Table 1 are data from the most recent College of American Pathologists survey for sample ACL-04 for the top four manufacturers’ reagent sets. Although the median values of three of the four methods are relatively close (43–48 units), the fourth is much different, and the CVs and ranges for each method are high. These data confirm that some variation in the ACA test is unavoidable and expected.

Drs. Hoefner and Yeo have asked that laboratories be informed when systematic changes occur. This is customary INOVA Diagnostics policy. In the case of the ACA IgM test, we and others did notice a shift in the reference preparation (Harris) that all manufacturers claim to use, but internal testing of our own patient panel did not reveal changes in the diagnostic result substantial enough, in light of the semiquantitative nature of the method, to warrant customer notification. Furthermore, a review of internal laboratory control values across several lots of reagents provided to us by Drs. Hoefner and Yeo during our attempts to resolve the situation again revealed no diagnostic changes in the semiquantitative results obtained with the reagent sets.

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Characteristics of the Cardiac Troponin I Assay on the Immulite 2000 Analyzer

To the Editor:

Recently, the Joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction proposed that “any amount of myocardial necrosis caused by ischemia should be labeled as an infarct” (1). The same committee agreed that a

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