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 \textsuperscript{TM} 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 \[ y = 1.34x + 5 \text{ GPL} \; \text{data not shown}. \] 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 mate-

![Fig. 1. Comparison of three lots of QUANTA Lite ACA IgM ELISA reagents. Reagent lots 170105, 170264, and 170355 used INOVA calibrators 123105A, 122809A, and 132240A, respectively. All data were generated from actual patient specimens; dashed lines represent the line of identity. Regression statistics: (A), \( y = 0.58x + 3 \text{ MPL} \; r = 0.992 \); slope (95% confidence interval), 0.523–0.647; y-intercept (95% confidence interval), 0.62–5.3 MPL; (B), \( y = 2.45x + 16 \text{ MPL} \; r = 0.95 \); slope, 2.06–2.78; y-intercept, −24 to 6.0 MPL; (C), \( y = 1.78x − 20 \text{ MPL} \; r = 0.987 \); slope, 1.26–3.30; y-intercept, −47 to 6.8 MPL.](image-url)
rials (data not shown). However, the
prior negative bias of the IgM was
overcompensated for; when the re-
vised reagent set (which contained
INOVA’s in-house secondary cali-
brators traceable to the GM-200 ma-
terial) was compared with the previ-
ut ion (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 cat-
egorical limits (i.e., negative, low,
medium, high positive) requires con-
sistency across reagent lots. The
INOVA product insert suggests that
results <12.5 MPL be classified as
negative, results ≥12.5 to 20 MPL be
classified as indeterminate, and re-
sults >20 MPL be reported as posi-
tive (with 20–80 MPL as low/me-
dium 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.

Reference
1. Wilson WA, Gharavi AE, Koike T, Lockshin MD,
Branch DW, Piette JC, et al. International con-
sensus statement on preliminary classification
criteria for definite antiphospholipid syndrome:
report of an international workshop. Arthritis

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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>
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<tr>
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<td>48</td>
<td>33</td>
<td>68</td>
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</tbody>
</table>

Representatives of INOVA Diagnos-
tics 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 micro-
well plate. In addition, the antigen
solid phase is complex, consisting of
both the phospholipid plus a neces-
sary cofactor, known as β2-glyco-
protein (β2-GPI), and the blocking
agent. Then there is the added prob-
lem of having to calibrate each re-
agent 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 stan-
dards in question, recommend that
results be reported in a semiquanti-
tative 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 re-
sults obtained 6 or more weeks apart
are necessary.

Shown in Table 1 are data from the
most recent College of American Pa-
thologists survey for sample ACL-04
for the top four manufacturers’ re-
agent sets. Although the median val-
ues 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 varia-
tion in the ACA test is unavoidable
and expected.

Drs. Hoefner and Yeo have asked
that laboratories be informed when
systemic changes occur. This is cus-
tomary 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 semiquanti-
tative nature of the method, to warrant cus-
tomer notification. Furthermore, a re-
view 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 diag-
nostic changes in the semiquanti-
tative 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 redef-
inition of myocardial infarction pro-
posed that “any amount of myocar-
dial necrosis caused by ischemia
should be labeled as an infarct” (1).
The same committee agreed that a