Characteristics of the cardiac troponin I assay on the Immulite 2000 analyzer

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providing details and we will investigate your claim.
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 ≥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.

References

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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>
</tr>
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<td>INOVA</td>
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<td>18.6</td>
<td>45</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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<tr>
<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 micro-well plate. In addition, the antigen solid phase is complex, consisting of both the phospholipid plus a necessary cofactor, known as β2-glycoprotein (β2-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 systemic 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
The maximum allowable imprecision at this decision limit has arbitrarily been set at a CV of 10% (1, 2), although it is not stated whether the 10% relates to total CV. Because an increasing number of nonstandardized commercial methods for the determination of cardiac troponin I (cTnI) are available, the analytical characteristics of every assay need to be established and published (1, 3). We describe the characteristics of the Immulite 2000 cTnI assay manufactured by Diagnostic Products Corporation.

The Immulite 2000 cTnI sandwich assay uses a monoclonal binding antibody and an alkaline phosphatase-conjugated polyclonal tracer antibody. Both antibodies recognize epitopes between amino acids 33 and 110 of cTnI. After incubation, excess conjugate is removed and chemiluminescent substrate is added. Chemiluminescence is proportional to the concentration of cTnI in the sample.

We assessed the detection limit of the assay by measuring 20 times a cTnI-free calibrator supplied by DPC. Each result was $<0.01 \mu g/L$. Therefore, the detection limit, defined as the signal 2 SD above the mean of the analyte-free calibrator, is $<0.02 \mu g/L$. To determine the 99th percentile of a control group, we analyzed heparinized plasma from 315 healthy volunteers from the outpatient clinic (approximately equal numbers of males and females, almost exclusively Caucasians). A total of 308 samples (98%) had cTnI concentrations $<0.01 \mu g/L$, and 7 samples contained 0.03–0.33 $\mu g/L$ cTnI. The 99% limit was calculated to be 0.32 $\mu g/L$. The authors of another study, with 117 participants, reported the 99% limit, measured in serum on the Immulite analyzer, as 0.48 $\mu g/L$ (4). Immulite serum cTnI values are 5–10% higher than those measured in heparin plasma (4, 5).

In accordance with the NCCLS EP5-T2 guideline (6), we analyzed five pools of heparinized plasma in duplicate once a day over 20 days and constructed an imprecision profile in the lower assay range. Our results (Fig. 1) are similar to those described previously for the Immulite analyzer (4, 5). A total CV of 10% was reached at a concentration of 0.7 $\mu g/L$. However, considering the 99th percentile found in the reference population, we arbitrarily chose a cutoff value of 0.4 $\mu g/L$ at a CV of 12% for routine clinical practice to increase the sensitivity of the assay for detection of minimal cardiac damage.

In summary, the Immulite 2000 cTnI assay is analytically suited for the triage of patients presenting with symptoms of myocardial damage. Like many other commercial troponin assays (7), its precision at low troponin concentrations is unsatisfactory, so future generations of the assay need to have improved analytical sensitivity.

We thank DPC Nederland (Breda, The Netherlands) for the cTnI reagent sets used in this study.

References