Our findings suggest the value of careful evaluation of new LightCycler genotyping assays and of controls for each genotype.

References


Siegfried Burggraf* 
Siegfried Kösel† 
Sabine Lohmann‡ 
Reinhard Beck‡ 
Bernhard Olgemöller‡

1 Diagnostic Laboratory Becker, Olgemöller und Kollegen Führichstrasse 70 81671 Munich, Germany

2 Roche Molecular Biochemicals Nonnenwald 2 82372 Penzberg, Germany

*Author for correspondence. Fax 49-89-450917-300; e-mail burggraf@labor-bo.de.

Heterophilic Antibody Interference with CARDIAC T Quantitative Rapid Assay

To the Editor:
Point-of-care troponin assays are promoted as providing rapid, reliable results to support best practice management of acute chest pain (1). We report a case of false-positive cardiac troponin T (cTnT) results that caused inappropriate clinical management.

A 46-year-old man consulted his general practitioner after 10 h of central chest pain and tingling in the left arm after a heavy fall. An electrocardiogram showed possible ST elevation. A repeat electrocardiogram in the local hospital showed sinus rhythm and an early repolarization pattern. Whole-blood cTnT (lithium heparinate) was increased both on admission and the next day in a CARDIAC T Quantitative Rapid Assay (third generation; Roche Diagnostics). The patient was a moderately heavy smoker, was mildly hypercholesterolemic, and had a family history of ischemic heart disease. The patient was transferred to the tertiary hospital where a coronary angiogram and plasma (lithium heparinate) was increased both on admission and the next day in a CARDIAC T Quantitative Rapid Assay (third generation; Roche Diagnostics) were normal (Table 1). Creatine kinase and lactate dehydrogenase concentrations in the two samples taken at the local hospital and two samples taken at the tertiary hospital were within the appropriate reference intervals (data not shown). The patient was diagnosed with presumed musculoskeletal chest pain.

The CARDIAC T sandwich assay uses a test strip and two murine monoclonal antibodies to human cTnT. A reader records the intensity of the reflectance signal (2). Calibration is against the Elecsys cTnT assay calibrators. Cross-reactivity with skeletal TnT is ~0.003% (1). The T STAT method uses the same monoclonal antibodies, with human recombinant cTnT for calibration; it is standardized against the second-generation Elecsys Tropo-
Table 1. cTnT results obtained by the CARDIAC T Quantitative Rapid and Troponin T STAT methods.

<table>
<thead>
<tr>
<th>District hospital</th>
<th>CARDIAC T Rapid</th>
<th>T STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 29, 2001, at 1140 (sample 1); whole blood (lithium heparinate)</td>
<td>0.59</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>March 30, 2001, at 1330 (sample 2); whole blood (lithium heparinate)</td>
<td>0.41</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Central laboratory</th>
<th>CARDIAC T Rapid</th>
<th>T STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 30, 2001, at 2030 (plasma)</td>
<td>0.36</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>March 31, 2001, at 0800 (plasma)</td>
<td>0.36</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Plasma from sample 1</td>
<td>0.49</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Plasma from sample 2</td>
<td>0.52</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Patient whole blood (lithium heparinate); collected May 4, 2001, at 1405 (sample 3)</td>
<td>0.50</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Patient serum; collected May 4, 2001, at 1405</td>
<td>0.49</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Plasma from sample 3</td>
<td>0.39</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>200 μL of plasma + 25 μL of mouse serum</td>
<td>0.17</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>200 μL of plasma + 50 μL of mouse serum</td>
<td>&lt;0.1 (0.05–0.09)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>200 μL of plasma + 75 μL of mouse serum</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

*Values in parentheses are upper reference limits.

antibody interference. The prevalence of HAMA interference in sandwich monoclonal immunoassays, reported variously as <1% to >40%, is poorly understood and may depend on the population studied (3). Such interference has been reported for troponin I (4) and the second-generation Elecsys cTnT assay (3). We speculate that the HAMA interference our patient showed with the CARDIAC T assay may be attributable to either inadequate formulation of the anti-HAMA additives or a matrix effect of the fleeces on the sample–reagent interactions. False-positive cTnT results have the potential of causing serious clinical mismanagement. Until the nature and prevalence of HAMA interference with the CARDIAC T Assay are better understood or eliminated, we recommend that positive cTnT results inconsistent with other clinical information be confirmed by the T STAT method or by other cardiac markers.

References

Graham H. White1* Philip A. Tideman2

Departments of 1Medical Biochemistry and 2Cardiac Services Flinders Medical Centre Flinders Drive

*Author for correspondence. Fax 61-8-8204-4466; e-mail Graham.White@fmc.sa.gov.au.

Representatives of Roche Diagnostics respond:

To the Editor:

In their letter, Drs. White and Tideman came to the conclusion that “the third-generation CARDIAC T assay is susceptible to positive heterophilic mouse antibody interference”. In our response we want to comment on this conclusion and the reported findings.

CARDIAC T Quantitative contains a murine monoclonal antibody (MAK33-IgG1) as a human anti-mouse antibody (HAMA) blocking agent. The efficiency of this HAMA blocking antibody in CARDIAC T Quantitative has been repeatedly tested with heparinized blood samples where plasma was exchanged by two commercial HAMA sera (cat. nos. 1767275 and 1779940; Roche Diagnostics, Mannheim, Germany). It therefore is extremely unlikely that the reported results are a HAMA interference phenomenon. Because mouse serum, which was added by Drs. White and Tideman to the patient plasma, contains many possible “blocking agents”, it might be that another non-HAMA-type interferent was blocked by the addition of the mouse serum. The authors point out in their letter that the patient’s serum yielded two precipitin bands against mouse serum when tested by counter immunoelectrophoresis. A single band was observed against mouse IgG. This suggests that the other band did not react to mouse IgG and therefore was not a HAMA. Because the CARDIAC T Quantitative assay contains a specific anti-HAMA blocking agent as described above, it is most likely that the interference originates with the non-HAMA band. Investigations on clarifying this hypothesis are in progress.

In addition, a positive control experiment with a sample from a patient with confirmed acute myocardial

Bedford Park
South Australia 5042, Australia