analysis time, use of chemicals, and technical equipment.

Reference


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Serum and Plasma Samples for ACS:Systems Cardiac Markers

To The Editor:
The ACS:180® immunoassay system (Bayer Diagnostics) is an automated random analyzer that uses acridinium ester-based chemiluminescence detection. The architecture of the cardiac marker assays is a heterogeneous sandwich format, using monoclonal anti-marker antibody immobilized onto paramagnetic particles (capture) and polyclonal goat anti-human myoglobin, creatine kinase MB isoenzyme (CK-MB), or cardiac troponin I (cTnI) antibody labeled with acridinium ester (detector). Its processing time (~20 min if the analyzer is in standby mode) and precision (overall CV <5%) make the system suitable for emergency use (1). However, optimal performance of an assay system for cardiac markers depends not only on the instrument’s analysis time, but also on factors that affect the overall turnaround time, including the preanalytical steps necessary to prepare the sample (2). The use of plasma samples eliminates the extra time needed for clotting, thereby reducing the overall preanalytical time, but there can be significant differences between serum and plasma concentrations of cardiac markers (3). The aim of this study was to validate the possible use of two different anticoagulants (lithium heparinate and EDTA, tripotassium salt) for cardiac marker determinations on the ACS: 180 immunoassay system.

Twenty-five patients with acute myocardial infarction were studied. All gave informed consent. We collected three separate tubes (Sarstedt) to prepare serum, heparin plasma, and EDTA plasma in random order during one phlebotomy. ACS myoglobin, CK-MB, and cTnI assays were performed in duplicate on each of the 25 samples. The mean results on plasma samples were compared with the paired serum values, and the significance of the differences was evaluated (Wilcoxon rank-sum test).

The results obtained are shown in Fig. 1. ACS myoglobin concentrations in serum [median myoglobin value (range), 79 μg/L (24–607 μg/L)] and heparinized plasma [76 μg/L (24–588 μg/L)] showed no statistically significant difference (P = 0.0799). For plasma EDTA, the assay showed a mean change in myoglobin concentration [73 μg/L (25–601 μg/L)] of ~3.5% compared with paired serum samples (P = 0.0006). Results for serum [median cTnI value (range), 11.3 μg/L (0.4–43.6 μg/L)] and heparinized plasma [11.7 μg/L (0.4–47.7 μg/L)] also were not statistically different with the ACS cTnI test (P = 0.704). On the other hand, for plasma EDTA, this test showed a mean change in cTnI concentration [14.7 μg/L (0.5–49.3 μg/L)] of 30% compared with paired serum samples (P <0.0001). Finally, CK-MB values for heparin [35 μg/L (5–252 μg/L)] and EDTA samples [34 μg/L (5–243 μg/L)] averaged 18.6% and 14.6%, respectively, higher than those in serum samples [29 μg/L (4–199 μg/L); P <0.0001].

Although in our study serum and heparinized plasma results for myoglobin and cTnI showed no statistical differences, we cannot definitively conclude that there is no difference in results between these sample types because the statistical power of the performed tests was too low (0.38 for myoglobin and 0.05 for cTnI) to provide an acceptable degree of certainty. Furthermore, although there may not be a substantial overall mean difference in results obtained using heparinized plasma vs serum
for myoglobin and cTnI, there could be individual patients for whom plasma and serum do not yield comparable results. With regard to this point, looking at our individual findings and considering a difference greater than 10% as clinically significant, none of the myoglobin results exceeded this limit. Conversely, four cases for cTnI showed a difference greater than 10% for heparinized plasma samples compared with serum. Analysis of the values obtained (case no. 4, 3.75 vs 4.24 μg/L; case no. 7, 2.91 vs 3.36 μg/L; case no. 10, 0.36 vs 0.40 μg/L; case no. 18, 0.58 vs 0.67 μg/L for serum and heparin samples, respectively) suggests, however, that these differences can above all be ascribable to the analytical variation of the test. On the other hand, whereas the use of EDTA plasma for all three ACS cardiac markers and heparin for CK-MB caused a statistically significant bias, the correlation coefficients (≥0.99) suggested that, in these cases, plasma data may be acceptable for determining diagnosis of myocardial infarction and risk stratification, but at different decision cutoffs.

Although no claim is given in the manufacturer’s package insert on the possible use of EDTA samples, our data agree with those reported on the use of heparinized plasma for the ACS myoglobin and CK-MB assays. For the ACS cTnI assay, Bayer reports that heparinized plasma samples may produce a significant negative bias (up to 16%). This difference in comparison with our results can be explained by the use of different types of sampling tubes and/or different types of heparin. Unfortunately, both the collection tubes and the type of heparin used in the manufacturer’s experiments were not specified in the insert.

We thank Bayer Diagnostics for the loan of the instrument and the gift of reagents to carry out the study.

References
To the Editor: All Bayer ACS:180® and ADVIA® Centaur™ system chemiluminescent immunoassays are evaluated during product development for performance characteristics based on specimen collection tube type, anticoagulant, and sample handling. Recommendations are reported in the product inserts in the Specimen Collection and Handling section. The approved specimen types for the ACS:180/ADVIA Centaur CKMB II, Troponin I, and myoglobin assays are serum and heparinized plasma. EDTA plasma was evaluated and is not approved for these tests. In paired specimen studies of the CKMB II assay, we observed ~20% positive bias between heparinized plasma and serum. For the cTnI assay, we also report a negative bias of ~16% for heparinized plasma compared with serum. These biases are reported along with NCCLS sample handling and storage guidelines, which when rigorously followed minimize errors attributable to preanalytical variables (1). The authors are correct in noting that the small variations in the four cases cited for cTnI are well within the 95% confidence intervals of the reported precision of the test (total CV of 6.7% at 1.4 μg/L).

We also clearly recommend that heparinized plasma and serum from the same patient not be used interchangeably in testing, especially when repeat samples and serial profiling are the accepted testing protocol for confirming diagnosis. Good laboratory practice suggests that for an individual patient and study series, sample collection should be uniform regardless of whether there is a bias between sample collection types.

We support the National Academy of Clinical Biochemistry’s guidelines regarding the necessity of serial sampling of several cardiac markers at defined time intervals to most rapidly and accurately rule in or rule out acute coronary syndromes (2). As long as sample handling is the same on repeat testing, the linear relationship (r = 0.995) between serum and heparinized plasma makes any bias inconsequential with regard to obtaining an accurate diagnosis.

We currently list our usable range for the method as 1.0–960 mg/L.

Furthermore, Beckman Coulter is planning to introduce a higher sensitivity CRP assay in the near future. We do not want your readers to be confused by referring to the currently available IMMAGE method as “hs-CRP”.

Reference

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Measurement of Total Protein Is Not a Useful Inclusion in Liver Function Test Profiles

To the Editor: Biochemical and hematological pathology investigations often are requested using groupings or profiles of tests. In Australia, “liver function test” (LFT) is recognized by legislation (1) and consists of six or more tests, including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, (total) bilirubin, γ-glutamyl transpeptidase, lactate dehydrogenase, and (total) protein. Historically, although total protein has been included as a part of LFT, the total protein measurement is not specific for abnormal liver function or liver damage. We have examined whether the routine inclusion of total protein as a component of LFT leads to new diagnoses or alters patients’ clinical management.

At the time of this study, our laboratory primarily serviced a large, tertiary referral university teaching hospital. Total protein was measured using the Biuret method (2) on a Hitachi 747 analyzer (Boehringer-Mannheim/Roche Diagnostics). Using a computer, we examined 15 000 consecutive unselected requests, including ambulatory and hospitalized