Use of the BD Vacutainer Rapid Serum Tube Reduces False-Positive Results for Selected Beckman Coulter Unicel DxI Immunoassays

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Abstract

We investigated the use of serum samples from BD Vacutainer rapid serum tubes (RSTs; BD, Franklin Lakes, NJ) to reduce undetermined interferences contributing to false-positive immunoassay results in heparin plasma samples. Patients being evaluated for suspected myocardial infarction had specimens drawn into an RST in addition to the standard lithium-heparin plasma separator tube (PST). We measured 28 separate analytes in both specimens using immunoassay, electrochemical, and spectrophotometric methods. Higher results were observed in some PST specimens tested for troponin I, creatine kinase-MB isoenzyme, human chorionic gonadotropin, and thyroid-stimulating hormone. These discrepancies were investigated by repeating analyses after recentrifugation of both specimens. Reanalysis gave results for the PST specimens that were lower and agreed well with initial results from RSTs, suggesting false-positive rates of 10.8% for troponin I and about 2% for each of the other 3 analytes. Overall, specimens collected in RSTs had fewer false-positive immunoassay results than specimens collected in plasma separator tubes.

Concerns about the performance of cardiac troponin (Tn) assays have been reported previously for several platforms.1-3 We and others had observed that the Beckman Unicel DxI AccuTnI assay (Beckman Coulter, Miami, FL) sometimes yielded nonreproducible falsely increased results on some plasma samples.4 These Tn false-positives were identified by the finding of substantially lower values on reanalysis after recentrifugation in a high-speed microcentrifuge, suggesting that particulate matter could have a role in generating the erroneous result. However, while recentrifuging and repeating every sample with an elevated TnI concentration became an effective tool to prevent reporting false-positive results, the practice was laborious, difficult to automate, wasteful of reagents, and associated with a lengthy turnaround time.

Beckman Coulter (the AccuTnI assay vendor) and Becton Dickinson (the lithium heparin plasma separator tube [PST] vendor) suggested that the problem could be minimized by thoroughly mixing heparin anticoagulant into samples by repeatedly inverting the tube immediately after phlebotomy. However, adherence to “proper technique” was thought to be difficult to achieve with hurried staff because it slows the process of obtaining specimens and is especially awkward when multiple specimens must be drawn.

A solution that was independent of human factors was thought to have a greater chance of success. We hypothesized that switching from lithium heparin plasma to serum could ameliorate this problem by avoiding incomplete or delayed coagulation. In addition, platelets and cell fragments that do not readily penetrate separator gels could be removed...
by incorporation into the forming clot. Because standard protocols for preparing serum for testing include a lengthy coagulation period that is incompatible with the requirements of stat testing, we investigated whether the new Becton Dickinson Vacutainer rapid serum tubes (RSTs) (BD, Franklin Lakes, NJ) could provide rapid and reliable TnI results when assayed on the Beckman DxI platform and whether using these tubes in the routine hospital setting would reduce the rate of false-positives. Because we believed that several other immunoassays for low-concentration analytes might be affected similarly to TnI and also because we wanted to assess the validity of RSTs for routine chemistry analytes currently assayed using PSTs, we undertook a head-to-head comparison on clinical samples collected in our hospital using PSTs and RSTs.

Materials and Methods

Patient Population

This study was carried out using specimens from the emergency department and cardiac intensive care unit at a large, academic medical center with a significant proportion of trauma patients. As part of an ongoing clinical laboratory quality improvement project approved by the local institutional review board, a sample was drawn into an RST concurrently with the standard sample drawn into a PST in all patients in whom a TnI assay was ordered. Nursing staff were given a brief in-service session to orient them to the new RST, and they were provided with the manufacturer’s instructions for use. Specifically, caregivers were instructed to invert the RST 5 times immediately after phlebotomy. No specific instructions or reminders were given for the PSTs, although hospital policy dictates that these tubes should be inverted at least 5 times immediately after phlebotomy. The study was conducted during a period of 80 days and included samples from 226 patients.

Phlebotomy Tube Types

The Vacutainer RSTs with standard rubber stoppers and a serum separator gel (“orange top”) were provided by Becton Dickinson at no cost, and lithium heparin PSTs with Hemogard closures (“lime green top”) were purchased from Becton Dickinson. Immunoassay tests were performed on the Beckman Coulter Unicel DxI 800, all other assays performed on the Beckman Coulter Unicel DxC 800, and all samples were handled with a Beckman Power Processor total laboratory automation system. The workflow included the following: (1) logging in the PST sample for all requested clinical tests, including TnI; (2) using the Datalink middleware to add a panel of chemistry and immunoassay tests for the study, and (3) placing the RST and PST samples onto the automation line for routine processing.

Measured Analytes

Tests compared in this study included sodium, potassium, chloride, total carbon dioxide, anion gap, total calcium, glucose, blood urea nitrogen, creatinine, total protein, albumin, phosphorus, magnesium, total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, creatine kinase (CK)-MB isoenzyme (CK-MB), pancreatic amylase, lipase, ethanol, acetonitrilephene, TnI, thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). Data for all tests was deidentified and collected in a coded database. Because the lengthy assay time for TSH would have delayed the release of clinical test results, paired TSH assays were run after the initial test panels were completed and all clinical results reported.

Centrifugation Protocols

Routine centrifugation on the automation line is conducted for 4 minutes at 1,912g. For the purposes of this study, samples with TnI results more than an institutional decision value (≤0.4 ng/mL [≤0.4 µg/L]) and those with discrepant TSH, CK-MB, or hCG results were divided into aliquots in microcentrifuge tubes, recentrifuged in a separate microcentrifuge at 4,185g for 5 minutes, and reanalyzed directly on the DxI instrument.

Data Analysis

Data were tabulated on a Microsoft Excel spreadsheet (Microsoft, Redmond, WA), with statistical analyses and graphing performed with the R statistics language,5 MethComp package,6 and ggplot2 graphics package.7

Results

For the initial analysis, comparisons of results from the PSTs and RSTs were done. Most results agreed well between the RSTs and PSTs, with Pearson correlation coefficients generally greater than 0.95 and slopes close to 1.0. Supplementary data can be found at www.ajcp.com. For the TnI, CK-MB, hCG, and TSH assays, however, we sometimes observed random discrepancies between the initial RST results (RST1) and the initial PST results (PST1). Figure II, and the magnitudes of the discrepancies were often clinically significant Figure 2I.

All of these assays are for analytes normally present at very low concentrations, which would increase the relative contribution to total signal (and, thus, to apparent concentration) of any chemiluminescent noise generated by
analyte-independent mechanisms. To investigate possible interference from particulate matter as the cause of the discrepancies, sample pairs with discrepant results and adequate residual volume were recentrifuged off-line and reanalyzed (PST analysis 2 [PST2] and RST analysis 2 [RST2]). For TnI, CK-MB, and TSH, there was considerably better agreement overall between RST1 and RST2 samples, whereas the corresponding PST1 and PST2 samples showed poor reproducibility and often fell greatly in measured concentration after recentrifugation (Figure 3).

A single example of a presumptive RST1 hCG-false positive in a sample from a male patient (RST1, 239.6 mIU/mL [239.6 IU/L]; RST2, 1.3 mIU/mL [1.3 IU/L]) was observed, indicating that interferences may still be rarely observed with RSTs. In this specific case, the PST1 and PST2 results agreed well (PST1, 1.1 mIU/mL [1.1 IU/L]; PST2, 0.9 mIU/mL [0.9 IU/L]), consistent with the apparent randomness in the occurrence of discrepant results. Overall, the results for hCG are largely similar to those for TnI, CK-MB, and TSH in that there were several PST1 and RST1 discrepancies, with PST1 results generally much higher than RST1 results.

However, the frequency of PST1 and RST1 discrepancies for hCG at concentrations near the institutional reference interval limits (<5 mIU/mL = “negative”; 6-24 mIU/mL = “indeterminate”), as shown in Figure 1, is quite remarkable and indicates that this assay may be especially sensitive to interfering substances in plasma.

In most cases, the PST1 result was lower and agreed better with the RST1 result, indicating that the original PST result was likely a false-positive that could have been avoided with the RST. To estimate the prevalence of PST false-positive results, RST1 values were compared with PST1 values. The RST1 values were used as the true values based on the data depicted in Figure 3 that indicate greater reproducibility between RST1 and RST2 serum samples. Based on this method for false-positive identification, the use of RST serum samples would have reduced the false-positive result rate for TnI, CK-MB, hCG, and TSH (Table 1). It is important to note that no false-positive results would have been generated for RST TnI. Table 1 lists overall positive rates and apparent false-positive rates for PST1 samples at selected decision values.
Discussion

The differences between plasma and serum are well known in laboratory medicine. The use of plasma from PSTs has gained popularity primarily owing to the substantial reduction in turnaround times for stat clinical testing compared with the use of serum from standard serum tubes.

Unfortunately, the use of plasma places a greater reliance on proper phlebotomy technique to ensure adequate mixing of the patient blood specimen with the anticoagulant (in the case of this study, lithium heparin). The RST used in this study provides an alternative means of obtaining patient serum without the typical delay required for completion of clotting.

In the present study, we identified clinically significant reductions in false-positive results when using RSTs relative to PSTs for several common clinical immunoassays run on the Beckman Unicel DxI platform. Unfortunately, we have been unable to apply the results of this study to our primary goal of improving the accuracy and turnaround time of the AccuTnI assay because this assay was subsequently recalled due to an interplatform bias (http://www.fda.gov/downloads/Safety/Recalls/EnforcementReports/UCM217010.pdf; accessed February 2011). However, the AccuTnI assay is expected to be reintroduced in the near future after addressing the interplatform bias, but without resolving the problem of random false-positive results in some samples. We anticipate using RSTs for the reintroduced assay. In addition, we plan to use RSTs for other stat immunoassay tests to mitigate the risk of false-positive results. Despite reasonable correlations between PSTs and RSTs on most other tests we investigated, the lack of a clear advantage of RSTs over PSTs for those tests makes it difficult to justify the extra cost of RSTs (currently approximately 4 to 5 times higher than PSTs) for specimens that have no requests for TnI, CK-MB, hCG, or TSH.

It is important to note that this study presents comparison data from samples collected from patients undergoing clinical testing for suspected or confirmed myocardial infarction, instead of samples carefully obtained from healthy volunteers in a controlled laboratory environment. Thus, the results of this study may be more relevant to hospital-based clinical practices than standard intralaboratory correlations or “check out” studies. By using the serum samples generated from RSTs, we found a significant reduction in the number of erroneous results seen sporadically with plasma samples. As off-line recentrifugation of the errant PST samples yielded results concordant with those from RST samples, it is reasonable to assume that the observed interference was due to the random presence of particulate material that was not present in serum samples. Thus, this study indicates that RSTs yield fewer false-positive results than do PSTs for immunoassays performed on the low-concentration analytes TnI, CK-MB, hCG, and TSH.

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Rapid serum tubes for this study were provided by Becton Dickinson. Becton Dickinson did not participate otherwise in the study, the data analysis, or the writing of this report.

Figure 3 Results from comparisons of rapid serum tube (RST) initial results (RST,) vs second analysis results (RST,2) (triangles) and plasma separator tube (PST) initial results (PST,) vs second analysis results (PST,2) (circles) for creatine kinase-MB isoenzyme (CK-MB), human chorionic gonadotropin (hCG), troponin I (TnI), and thyroid-stimulating hormone (TSH). For each panel, the line of identity is shown as a dotted line. Axes are in units specific to each analyte. Conventional (Système International) units for the analytes are as follows: CK-MB, ng/mL; hCG, mIU/mL; TnI, ng/mL; TSH, mIU/L.

Table 1 Characterization of Results for PST1 Samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Decision Value</th>
<th>% Positive</th>
<th>Presumptive PST1 FP Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnI, ng/mL (µg/L)</td>
<td>0.4 (0.4)</td>
<td>18.9</td>
<td>2.27</td>
</tr>
<tr>
<td>TnI, ng/mL (µg/L)</td>
<td>0.04 (0.04)</td>
<td>43.0</td>
<td>10.8</td>
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<tr>
<td>CK-MB, ng/mL (µg/L)</td>
<td>5 (5)</td>
<td>33.5</td>
<td>2.00</td>
</tr>
<tr>
<td>hCG, mIU/mL (IU/L)</td>
<td>&gt;24 (24)</td>
<td>1.92</td>
<td>1.92</td>
</tr>
<tr>
<td>hCG, mIU/mL (IU/L)</td>
<td>&gt;5 (5)</td>
<td>2.58</td>
<td>0.53</td>
</tr>
<tr>
<td>TSH, mIU/L (mIU/L)</td>
<td>9.5 (5)</td>
<td>9.60</td>
<td>2.21</td>
</tr>
</tbody>
</table>

CK-MB, creatine kinase-MB isoenzyme; FP, false-positive; hCG, human chorionic gonadotropin; PST; plasma separator tube; TnI, troponin I; TSH, thyroid-stimulating hormone.
References


