**Effect of Collection Tube Type and Preanalytical Handling on Myeloperoxidase Concentrations**

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**BACKGROUND:** Myeloperoxidase (MPO) has shown potential as a marker for cardiovascular disease. Limited studies have been published with a variety of sample types, resulting in a wide range of MPO values. Little is known or understood about the impact of collection tube type and preanalytical handling of specimens for MPO determination.

**METHOD:** MPO concentration was determined by use of the ARCHITECT® MPO research use assay, which is currently under development. Samples were collected into multiple anticoagulant collection tubes from donors and patients presenting to the emergency department with symptoms of acute coronary syndromes. Whole blood was stored on ice or at room temperature before centrifugation as whole blood samples before centrifugation, as well as serum or plasma before analysis on the Abbott ARCHITECT system. In the clinical use of MPO, an important consideration in preanalytical handling conditions of the specimen is prevention of artificial release of MPO from the neutrophils in the samples, which may lead to falsely increased results. The current FDA-cleared product provides little to no information on this important aspect.

MPO concentrations were determined by use of a new prototype assay for MPO on the ARCHITECT instrument. The ARCHITECT MPO assay is an automated chemiluminescent microparticle immunoassay using Chemiflex® technology. MPO-specific monoclonal antibodies are used in a 2-step sandwich format. For this assay, total CVs of 2.6%–6.8% over a range of 277–3457 pmol/L have been reported. When tested on 3 lots the assay had a limit of detection of 20 pmol/L and a functional sensitivity of 125 pmol/L (total CV of 10%) (10, 11).

Normal donor samples used in this study were collected in accordance with an institutional review board–approved protocol, and sample donors gave informed consent. These samples were used in all experiments except for the direct correlation study. Surplus samples from patients presenting to the emergency department with chest pain suggestive of acute coronary syndrome were selected in the participating clinical laboratory after required testing was performed. Testing protocols for chest pain patients called for collection of matched samples in serum separator, K3EDTA, lithium heparin plasma separator, and citrate tubes. The anonymous use of surplus samples from emergency department patients for study purposes was regulated under the contract for treatment that is signed by every patient. Samples were centrifuged for 10 min at 1250g in accordance with blood collection tube manufacturers’ instructions.

**RESULTS:** Baseline sample concentrations were dependent on collection tube type as well as handling conditions. MPO concentrations were consistently higher in samples collected in serum and heparin plasma tubes than in samples in EDTA or citrate tubes. Spike recovery was acceptable in all sera and plasma tested, indicating that the increased MPO concentrations were not due directly to an anticoagulant interference.

**CONCLUSIONS:** The collection tube type and preanalytical handling are critical for accurate and consistent MPO measurement. The preferred anticoagulant and tubes are the EDTA or EDTA plasma preparation tube. MPO concentrations in samples collected in these tubes are stable before centrifugation as whole blood as well as plasma after processing.

Myeloperoxidase (MPO) is an enzyme found in leukocytes that catalyzes the formation of reactive oxidants, diffusible radical species, and nitric oxide–derived oxidants. MPO has been reported to be present in atherosclerotic plaques, is enriched in culprit lesions, and oxidizes LDL in the artery wall (1–3). Recent reports have linked increased concentrations of circulating MPO with increased risk for coronary disease, increased risk in patients with acute coronary syndrome (4–7), and clinical utility in the identification and prognosis of heart failure patients (8, 9).

As new markers are identified and assays developed for quantifying these markers, preanalytical and analytical variables that may affect the analyte or assay must be defined. We investigated the effects of preanalytical handling on measured MPO concentrations by evaluating blood collection tube acceptability and storage of whole blood samples before centrifugation, as well as serum or plasma before analysis on the Abbott ARCHITECT system. In the clinical use of MPO, an important consideration in preanalytical handling conditions of the specimen is prevention of artificial release of MPO from the neutrophils in the samples, which may lead to falsely increased results. The current FDA-cleared product provides little to no information on this important aspect.
Normal donor samples collected into 9 different tube types were evaluated: serum (glass and plastic), serum separator, K$_2$EDTA, K$_2$EDTA plasma preparation, lithium heparin (glass and plastic), lithium heparin plasma separator, sodium heparin, and sodium citrate tubes. The lowest concentration of MPO was found with EDTA (92 pmol/L) and citrate plasma (94 pmol/L), with the heparin samples (101–110 pmol/L) approximately 10% higher and the serum samples (145–192 pmol/L) up to 100% higher. Statistically (Wilcoxon test) there was no difference in value between EDTA and citrate, whereas the difference between EDTA and any of the heparin or serum samples was statistically significant ($P<0.001$). To further investigate this anticoagulant effect directly on the assay, we performed a spike recovery experiment, adding 2400 pmol/L native MPO to an aliquot of each sample. The recoveries ranged from 99% to 102% with the ARCHITECT assay and showed no effect of the anticoagulant on the MPO analyte itself. (See Table 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol54/issue6.)

We investigated the effects of storage temperature and time before centrifugation of whole blood for EDTA, EDTA plasma preparation, lithium heparin plasma separator, and serum separator tubes and evaluated for stability at room temperature, 2–8 °C and after multiple freeze-thaw cycles after centrifugation and removal of the supernatant. Room temperature storage was evaluated for time periods of 0, 3.5, 24, and 48 h; 2–8 °C storage for time periods of 0, 1, 2, 5, and 8 days; and freeze-thaw stability was evaluated after up to 3 cycles. Less than 10% differences were found after storage of samples at room temperature for 2 days, after storage at 2–8 °C for 8 days, and after 3 freeze-thaw cycles for all sample types post centrifugation. (See Table 2 in the online Data Supplement.)

Fifty matched sets of samples from patients presenting with symptoms of acute coronary syndrome were evaluated. All samples were stored at room temperature for no more than 60 min before centrifuga-
tion, except the EDTA samples that were stored at 2–8 °C as whole blood for up to 24 h before centrifugation. Separated serum and plasma samples were stored at 2–8 °C until tested. The mean and median sample concentrations were dependent on collection tube type (Table 1A). The citrate and EDTA plasma values were similar and substantially lower than the values from serum and lithium heparin samples. The correlation of the samples relative to EDTA is shown in Table 1B. (See Fig. 1, A–C in the online Data Supplement.)

Previous investigations in other clinical settings have demonstrated the effect of heparin and citrate on MPO values, supporting the data we report here (12, 13). A recent report by Chang et al. discussed the effect of temperature on storage of heparin samples for MPO measurement (12). During a 6-h storage period for whole blood heparin samples at room temperature, Chang et al. observed a dramatic increase in MPO concentrations compared to those of samples stored on ice. Further comment was made on the preparation of calibrator material using plasma from heparinized whole blood samples that had been allowed to sit at room temperature for 3 hours to obtain increased MPO concentrations compared to those of samples stored on ice. A more recent report (6) provides details of careful sample handling with the use of heparin tubes. In these study patients the median MPO concentration was 1234 pmol/L and the 99th percentile of normal concentration was 866 pmol/L.

During hemodialysis, polymorphonuclear cells and platelets degranulate, a process that is thought to be calcium dependent and can be abolished by citrate anticoagulation (13). Comparison of heparin with citrate anticoagulation in samples collected during hemodialysis showed that MPO concentrations (as a surrogate marker for polymorphonuclear cell degranulation) increased immediately in patients receiving heparin for anticoagulation but remained stable in patients receiving citrate for anticoagulation.

In a study investigating adjuvant therapy during percutaneous coronary intervention (14), the administration of unfractionated heparin increased the concentrations of MPO, whereas no effect was seen with bivalirudin. With the use of an in vitro assay, these investigators showed that heparin stimulated MPO release from neutrophils during neutrophil activation.

Thus, to ensure overall reliability of results for MPO measurement, selection of the sample collection tube type is very important. The anticoagulant itself does not appear to interfere in the assay, as demonstrated by excellent spike recovery, but the preanalyti-

<table>
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<th>Tube type</th>
<th>Mean MPO, pmol/L</th>
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<th>Maximum MPO, pmol/L</th>
</tr>
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<tbody>
<tr>
<td>Serum</td>
<td>918</td>
<td>644</td>
<td>188</td>
<td>4882</td>
</tr>
<tr>
<td>EDTA</td>
<td>314</td>
<td>149</td>
<td>31</td>
<td>3791</td>
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<tr>
<td>Heparin</td>
<td>841</td>
<td>493</td>
<td>122</td>
<td>8252</td>
</tr>
<tr>
<td>Citrate</td>
<td>301</td>
<td>128</td>
<td>44</td>
<td>3501</td>
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B. Tube type correlation (Passing-Bablok regression) for samples from 50 patients.

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<tr>
<th>Slope Intercept</th>
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<tr>
<td>EDTA vs citrate</td>
<td>0.87</td>
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<tr>
<td>EDTA vs heparin</td>
<td>3.44</td>
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<tr>
<td>EDTA vs serum</td>
<td>4.14</td>
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In the study using frozen serum, the median MPO concentrations were 10-fold higher than those in the study using frozen plasma (1979 pmol/L vs 198 pmol/L). The reports of these studies provided no information on the preanalytical handling of the samples. In light of our results, it is evident that MPO concentrations in serum are higher owing to the leakage of MPO from leukocytes. A more recent report (6) provides details of careful sample handling with the use of heparin tubes. In these study patients the median MPO concentration was 1234 pmol/L and the 99th percentile of normal concentration was 866 pmol/L.

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cal handling during the clotting of serum and preparation of heparin plasma allows for leakage of MPO out of leukocytes, causing an increase in serum or plasma MPO. Our data indicate that EDTA inhibits this leukocyte leakage at room temperature and therefore should be the preferred anticoagulant for samples collected for MPO determination.

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References


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