Multicenter Evaluation of an Automated Assay for Troponin I

Denise Uettwiller-Geiger, 1 Alan H.B. Wu, 2 Fred S. Apple, 3 Anthony W. Jevans, 4 Per Venge, 5 Marilyn D. Olson, 6 Claude Darte, 6 David L. Woodrum, 6 Sean Roberts, 6 and Stephen Chan 6*

Background: Cardiac troponin I (cTnI) is a powerful tool to aid in the diagnosis of myocardial infarction and cardiac muscle damage. We describe an assay that overcomes problems of early assays that were often affected by cTnI degradation, assay interference, poor sensitivity, and imprecision.

Methods: The analytical performance of the Access® AccuTnITM assay (Beckman Coulter) was evaluated at five institutions. Controls, zero calibrator, and diluted patient samples were used to determine precision, detection limit, functional sensitivity, and linearity. The 97.5 and 99 percentiles of a reference population were determined. Common interferents and heterophilic patient samples were tested. Equimolarity was determined by assaying samples with various ratios of free and complexed cTnI. Matched samples drawn into serum, EDTA, lithium heparin, and sodium heparin sample tubes were compared.

Results: Total imprecision (CVs) was 4.0–8.8% between 0.40 and 31 μg/L cTnI. The detection limit was <0.01 μg/L. The 97.5 percentile upper reference limit (URL) was 0.03 μg/L (CV = 20%), and the 99 percentile URL was 0.04 μg/L (CV = 14%). Total CVs of 10% and 20% were seen at and above 0.06 and 0.03 μg/L, respectively. The assay was linear to >60 μg/L and not affected by common assay interferents. An equimolar response was observed with free, complexed, phosphorylated, and dephosphorylated forms of cTnI. Results were 4% lower in serum and 14% lower in EDTA plasma than in lithium heparin plasma (P <0.01), independent of cTnI concentration.

Conclusion: AccuTnI is a sensitive and precise assay for the measurement of cTnI.

© 2002 American Association for Clinical Chemistry

Previously, the WHO defined myocardial infarction (MI)7 by the presence of a combination of two of three clinical symptoms: chest discomfort, an increase in cardiac-associated enzymes, and/or a typical electrocardiogram pattern involving the development of Q-waves or ST-segment elevations (1). The high specificity of cardiac troponins for myocardial necrosis caused by prolonged ischemia has led the National Academy of Clinical Biochemistry (NACB), IFCC, and the joint committee of the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) to reexamine the definition of MI to recommend that any increase in cardiac troponin [above the upper reference limit (URL) of a reference population] be viewed as indicative of myocardial injury in the clinical setting of ischemic MI (2–5). As such, treatment decisions may be made on the basis of relatively small increases in cardiac troponin. This necessitates the use of highly specific, sensitive, and precise troponin assays.

The design of the AccuTnITM assay for cardiac troponin I (cTnI) and the criteria for analytical evaluation were tailored in accordance with NCCLS guidelines (6–10) and NACB, IFCC (2–4), and ESC/ACC recommendations (5). A cTn assay should include the use of antibodies directed to stable cardiac-specific epitopes and should recognize

7 Nonstandard abbreviations: MI, myocardial infarction; NACB, National Academy of Clinical Biochemistry; ESC, European Society of Cardiology; ACC, American College of Cardiology; URL, upper reference limit; (95% confidence interval appears as ± x); cTnC, cTnI, and cTnT, cardiac troponin C, I, and T; HAMA, human anti-mouse antibody; RF, rheumatoid factor; LiHep, lithium heparin; cTnICT, cardiac troponin ICT ternary complex; NaHep, sodium heparin; AMI, acute MI; AA, amino acid; and cTnIC and cTnIT, cardiac troponin IC and IT binary complexes.
all the molecular forms of cTnI in an equimolar manner (3). The assay must also be free of heparin interference and interference from blood constituents, common drugs, heterophilic antibodies, human anti-mouse antibodies (HAMAs), and rheumatoid factors (RFs) (3,8). Finally, low-end precision is critical and should be evaluated in accordance with NACB, IFCC, and ESC/ACC recommendations. The purpose of this study was to characterize the analytical performance of the Access® AccuTnI assay in regard to these criteria.

Materials and Methods

ASSAY METHOD

AccuTnI is a new assay for cTnI that uses two new murine monoclonal antibodies, one (directed to amino acids 24–40) is conjugated to alkaline phosphatase, and the other (directed to amino acids 41–49) is coated on paramagnetic particles. The Access Immunoassay System is a random access, bench-top analyzer that has been described in detail elsewhere (11). Time to first result for this assay is 12 min, with successive results in 36-s increments.

CALIBRATION CURVES AND PRECISION

Calibration curves. The assay system was calibrated according to manufacturer’s instructions throughout the course of the evaluation. Calibrator curve precision and stability were determined from these data.

Precision. On the basis of NCCLS guideline EP5-A (6), each of the five institutions tested three commercial controls and two manufacturer’s serum pools in triplicate in two runs/day for 20 days, using two lots of reagents. Within-run and total imprecision were calculated for each control at each site.

FUNCTIONAL SENSITIVITY, DETECTION LIMIT, AND URL

Functional sensitivity was determined as described by Spencer et al. (12) for thyroid-stimulating hormone and as modified by Yeo et al. (13) for cTnI or cardiac troponin T (cTnT). A pool of lithium heparin (LiHep) plasma samples with increased cTnI was diluted in a pool of normal LiHep plasma until the recoveries of the dilutions were no longer distinguishable from zero. The detection limit, defined as the concentration of cTnI corresponding to a signal 2 SD above the mean of 10 replicates of the zero calibrator, was determined three times at each of five institutions.

The 97.5 and 99 percentile URLs were determined by testing 254 apparently healthy individuals (88 males and 166 females; age range, 19–88 years). Of the 254 reference control individuals, 14% were same-day surgery patients (collected before surgery), 20% were healthy volunteers (out-of-hospital controls), and 66% were outpatients. The same-day surgery patients and outpatients were from noncardiac-related clinics, e.g., dermatology. There was no known cardiac involvement from the enrolled participants at the time the blood was collected. The study was performed in compliance with the requirements of each institution’s Institutional Review Board (14).

CROSS-REACTIVITY

Analytical specificity was determined by comparing a serum sample with a low but measurable concentration of cTnI to aliquots of a sample to which 1000 µg/L skeletal TnI, cardiac troponin C (cTnC), cTnT (Hytest Ltd.), recombinant cTnT (gift from James Potter, University of Miami, Miami, FL), actin, myosin, troponymosin (Vital Products), creatine kinase MB isoform (Fitzgerald Industries), and myoglobin (Cortex Biochem) had been added.

RECOGNITION OF FREE AND COMPLEXED TROPOinin FORMS

Recombinant fragments of cTnI (Spectral Diagnostics), synthetic peptides (Genemed Synthesis), and phosphorylated and dephosphorylated cardiac troponin ICT ternary complexes (cTnICT; Hytest Ltd.) were serially diluted in normal human serum and tested. In a second experiment, increasing amounts of cTnC or cTnT (up to 200 µg/L) were added to LiHep-plasma and serum samples, each containing 20 µg/L purified free cTnI, and incubated for 1 h at room temperature before being tested (15). Additionally, increasing amounts of cTnT (up to 800 µg/L) were added to samples containing 20 µg/L cTnI and 80 µg/L cTnC and incubated before being tested.

INTERFERING SUBSTANCES

Various blood components and cardiac-related drugs obtained from commercial sources were tested according to NCCLS EP7-P Appendix C guideline (8). Results for samples enriched with these possible interferents that were within 5% of the results for control samples were considered acceptable. In addition, samples from 254 controls, 22 HAMA samples obtained from patients treated therapeutically with mouse monoclonal antibodies, 101 samples containing heterophilic antibodies, and 17 RF samples were tested for assay interferences (Scantibodies and internal sources).

LINEARITY ON DILUTION

Four LiHep-plasma samples with increased cTnI were used to prepare five intermediary dilutions (80%, 60%, 40%, 20%, and 10%) of each specimen in assay sample diluent as described in NCCLS guideline EP6-P (7).

SAMPLE TYPE

We compared 89 matched samples drawn into serum, EDTA plasma, LiHep plasma, and sodium heparin (NaHep) plasma tubes. Recoveries for fresh LiHep-plasma samples and samples frozen for at least 3 days at −20 °C were also compared.

METHOD COMPARISON

The Access AccuTnI was compared with the Ortho Vitros ECi™ (16), Abbott AxSYM™ (17), and Dade-Behring
Dimension RxL™ (18) cTnI assays8 using LiHep-plasma samples selected to represent the range of values likely to be encountered in clinical practice. The comparison data were analyzed by Deming regression, by Bland–Altman bias plots, and by concordance analyses. Two-by-two concordance analysis at the acute MI (AMI) cutoffs published in the manufacturers’ inserts was performed.

**HIGH-DOSE HOOK EFFECT**
Purified human cTnICT (BioStrategic Solutions, Inc.) was added to pooled human serum at concentrations up to 14 000 μg/L and tested.

**STATISTICAL ANALYSIS**
Sample type and method-comparison regression analyses were performed according to the Deming method described by Mandel (19). Linearity on dilution was determined by least-squares regression analysis (7). The agreement between two methods was assessed by the method described by Bland and Altman (20). Probabilities for t-tests were calculated using Analyze-it Software for Microsoft EXCEL, Ver. 1.5 (April 2000; Analyze-it Software, Ltd.).

**ROLE OF THE SPONSOR**
Beckman Coulter participated in the study design, data collection and analysis, and manuscript preparation.

**Results**

**CALIBRATION CURVES AND PRECISION**
Calibration curves stored for up to 79 days were compared with fresh calibration curves. Calibration stability observed for the two lots of reagent were the same. Values for individual control samples recovered from stored calibration were 91–104% of the values recovered from fresh calibration and were not statistically different (P >0.4). The maximum interlaboratory within- and between-run imprecisions (CVs) of the five concentrations of non-zero calibrators were 7.7% and 10%, respectively. The within-run and total imprecisions (CVs) of the five controls between 0.40 and 31 μg/L were 2.6–4.6% and 4.0–8.8%, respectively, across two lots of reagents and five institutions. Total interlaboratory CVs were 6.1–7.2% for all the controls across institutions (Table 1).

**FUNCTIONAL SENSITIVITY, DETECTION LIMIT, AND UPPER REFERENCE LIMIT**
The functional sensitivity, defined as the concentration with a total imprecision (CV) ≤20%, ranged from 0.02 to 0.04 μg/L with a median of 0.03 μg/L. At a total imprecision (CV) of 10%, the functional sensitivity ranged from 0.05 to 0.11 μg/L with a median of 0.06 μg/L. The detection limit ranged from 0.001 to 0.018 μg/L with a median of 0.004 μg/L (Table 2). At the 97.5 and 99 percentiles for 254 control individuals, the URLs for

---

<table>
<thead>
<tr>
<th>Table 1. Precision data from five institutions with two lots of reagents over 40 analytical runs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>Total interlaboratory precision</td>
</tr>
</tbody>
</table>

---

*Given as mean measured concentrations and total CVs.

---

<table>
<thead>
<tr>
<th>Table 2. Access AccuTnI assay detection limit, functional sensitivity, and URLs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection limit, μg/L</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Functional sensitivity, μg/L</td>
</tr>
<tr>
<td>At 20% CV</td>
</tr>
<tr>
<td>At 10% CV</td>
</tr>
<tr>
<td>CV, % At 97.5 percentile URL (0.03 μg/L)</td>
</tr>
<tr>
<td>At 99 percentile URL (0.04 μg/L)</td>
</tr>
</tbody>
</table>

---

*All trademarks are properties of the respective owners.
AccuTnI were 0.03 and 0.04 μg/L, respectively (Fig. 1). The median total imprecisions (CV) corresponding to the 97.5 and 99 percentiles were 20% (range, 15–25%) and 14% (range, 11–18%), respectively.

CROSS-REACTIVITY
The cross-reactivities of the AccuTnI assay to cTnC, cTnT, recombinant cTnT, actin, myosin, tropomyosin, creatine kinase MB isoenzyme, and myoglobin were ≤0.01%, and the cross-reactivity to skeletal TnI was 0.034%.

RECOGNITION OF FREE AND COMPLEXED TROPONIN FORMS
A recombinant fragment containing amino acid (AA) sequence 1–99 was quantitatively recovered by the assay, whereas a recombinant fragment containing AA sequence 55–210 recovered <1% of AA sequence 1–99. A commercially produced synthetic peptide covering AA sequence 20–79 was also quantitatively recovered by the assay. The ratio of the recoveries of dephosphorylated and phosphorylated cTnI forms was 93% for serial dilutions through the analytical range. The titration of up to 200 μg/L cTnC into 20 μg/L free cTnI produced recoveries of 100% in LiHep plasma and 117% in serum. The titration of up to 200 μg/L cTnT into 20 μg/L free cTnI produced recoveries of 94% in LiHep plasma and 99% in serum. The titration of up to 800 μg/L cTnT into the cardiac troponin IC binary complex (cTnIC) gave recoveries of 96% in LiHep plasma and 97% in serum. The AccuTnI equally measured free cTnI and cTnI complexed with cTnT or cTnC (binary complex) or complexed with cTnC and cTnT (ternary complex) in both LiHep plasma and serum.

INTERFERING SUBSTANCES
One of 254 samples from the control individuals, 2 of 22 HAMA samples, 2 of 101 samples containing heterophilic antibodies, and none of 17 RF samples tested above 0.04 μg/L, the 99 percentile URL. Of the samples that tested above 0.04 μg/L, only one HAMA sample (0.07 μg/L) and one heterophilic antibody sample (0.24 μg/L) tested above 0.06 μg/L, the functional sensitivity at a CV of 10%. Incubation of the sample containing heterophilic antibodies in a Scantibodies HBT tube caused the cTnI value to decrease from 0.24 to 0.07 μg/L, indicating that the initial response was most likely attributable to interference from the heterophilic antibodies. None of the various blood components (bilirubin, human serum albumin, hemoglobin, triglycerides, fibrinogen, and alkaline phosphatase) or cardiac-related drugs (abciximab, acetaminophen, allopurinol, amoxrol, ampicillin, ascorbic acid, aspirin, atenolol, caffeine, captorpl, cinnarizine, cocaine, diclofenac, digoxin, dopamine, erythromycin, furosemide, ibuprofen, low-molecular weight heparin, methyldopa, nifedipine, nitrofurantoin, nystatin, oxytetracycline, phenytoin, propranolol, quinidine, sodium heparin, theophylline, trimethoprim, and verapamil) tested interfered with the assay; all results were within 5% of controls.

LINEARITY ON DILUTION
Dilution of the four samples with initial values of 0.82, 2.39, 13.51, and 60.48 μg/L provided mean recoveries of 106%, 100%, 98%, and 94%, respectively. Slopes of the lines of observed vs expected values ranged from 0.984 (±0.020) to 1.028 (±0.062), and the correlation coefficient ranged from 0.999 to 1.000. The slopes were not significantly different from 1.0 (P >0.1).

SAMPLE TYPE
On average, recoveries for serum, EDTA-plasma, and NaHep-plasma samples were 4%, 14%, and 2% lower than the recoveries for the matched LiHep-plasma samples, respectively (n = 89; P <0.01 for test of mean difference). Sample type comparisons yielded the following: EDTA = 0.895(±0.011)serum + 0.883 (r = 0.999; Sd= 0.471); Nehep = 0.978(±0.007)LiHep - 0.043 (r = 0.999; Sd = 0.486); serum = 0.961(±0.008)LiHep - 0.188 (r = 0.999; Sd = 0.607); EDTA = 0.864(±0.009)LiHep - 0.049 (r = 0.999; Sd = 0.626). All sample types exhibited a good correlation to paired fresh heparin-plasma samples.

Recoveries for LiHep-plasma samples (cTnI concentration range, 0.03–97.54 μg/L) that had been frozen for a minimum of 3 days before thawing were 5% lower than recoveries for matched fresh samples: frozen = 0.952(±0.007)frozen + 0.129 (r = 0.999; Sd = 0.712; n = 169; P <0.01 for test of mean difference). When the range was truncated to 0.03–4.93 μg/L, the recovery for the frozen samples was 2% higher than that of the matched fresh samples: frozen = 1.019(±0.018)frozen - 0.011 (r = 0.999; Sd = 0.140); n = 115; P = 0.2 for test of mean difference).

METHOD COMPARISON
Comparisons of different assay systems produced a wide range of slopes and correlation coefficients, confirming
the large degree of heterogeneity from one manufacturer’s product to another product (Fig. 2). Although the three methods that were compared showed significant differences in slope ($0.183–1.109$), there was relatively good correlation ($r = 0.946–0.980$) when patient sample recoveries across the full range of the assays were compared. When the cTnI range was truncated to $0–1.5 \mu g/L$ (by the AccuTnI method), the correlations between methods were different. Comparisons of the AccuTnI range of $0–1.5 \mu g/L$ with the RxL, ECI, and AxSYM methods by bias plots assessed the methods’ agreement at the concentration range of the clinical decision points (Fig. 3). The total concordance at the AMI cutoffs between the AccuTnI ($0.5 \mu g/L$) and the RxL ($1.5 \mu g/L$), ECI ($0.8 \mu g/L$), and AxSYM ($2.0 \mu g/L$) methods was $94\%$ ($\pm 3.75\%$), $91\%$ ($\pm 3.20\%$), and $91\%$ ($\pm 4.93\%$), respectively. The high concordance demonstrated good agreement in clinical AMI diagnosis between the AccuTnI and the commercial methods evaluated.

**Discussion**

The Access AccuTnI assay is an accurate and robust assay that provides reproducible results within an assay and from assay to assay, lot to lot, and site to site. The assay dynamic range is $0.01–100 \mu g/L$. The 97.5 percentile and the 99 percentile URLs were $0.03$ and $0.04 \mu g/L$, respectively. The total imprecision (CV) at the 97.5 and 99 percentiles was $20\%$ and $14\%$, respectively. The lowest concentrations with a total imprecision (CV) of $10\%$ and $20\%$ ("functional sensitivities") were $0.06$ and $0.03 \mu g/L$, respectively. Cross-reactivity and assay interference were minimal. The assay gave an equimolar response to free cTnI, the various complexed forms of cTnI, and phosphorylated and dephosphorylated cTnI complexes. No statistically significant bias in linearity was observed when samples were diluted. Serum and heparin-plasma samples types were substantially equivalent, and EDTA plasma showed an average $-14\%$ bias.

The AccuTnI assay uses two anti-human cTnI monoclonal antibodies that react with epitopes located between amino acids 24 and 49. The targeting of this cTnI AA region reflects a balance between the needed specificity to cardiac-derived troponin I and stability to in vivo and in vivo conditions.

**High-dose hook effect**

No high-dose hook effect was seen with concentrations tested up to $14 000 \mu g/L$. 

**Fig. 2.** Method comparison using LiHep-plasma samples. For comparisons across the full ranges of the comparison methods (top panels): AccuTnI $= 0.932(\pm 0.029)$RxL $- 1.039 (r = 0.980; S_{xy} = 1.810; n = 157)$; AccuTnI $= 1.109(\pm 0.031)$ECi $- 0.473 (r = 0.968; S_{xy} = 2.135; n = 316)$; AccuTnI $= 0.183(\pm 0.010)$AxSYM $+ 0.062 (r = 0.946; S_{xy} = 0.658; n = 128)$. For comparisons using LiHep-plasma samples containing $0–1.5 \mu g/L$ cTnI (bottom panels): AccuTnI $= 0.953(\pm 0.039)$RxL $- 0.032 (r = 0.941; S_{xy} = 0.129; n = 92)$; AccuTnI $= 0.747(\pm 0.087)$ECi $+ 0.100 (r = 0.733; S_{xy} = 0.296; n = 134)$; AccuTnI $= 0.153(\pm 0.020)$AxSYM $+ 0.104 (r = 0.835; S_{xy} = 0.243; n = 54)$.
In vitro degradation and to modifications to the cTnI molecule (21–25). In vitro stability of matched serum and heparin-plasma sample types stored up to 48 h has been reported by Venge et al. (26), who found recoveries for stored samples within 10% of baseline. Ser-23 and Ser-24 are susceptible to phosphorylation (27). Phosphorylation of cTnI was shown to have no effect on the AccuTnI assay. After blood collection, cTnI may oxidize to form intramolecular disulfide linkages between Cys-80 and Cys-97 (28, 29). We did not test the effects of oxidation. However, analysis of the antibody pair binding region and the stability data reported by Venge et al. (26) imply that this should not be a significant source of assay interference.

cTnI is released into the circulation predominantly as cTnIC, and in smaller amounts as free cTnI and ternary troponin complex cTnICT (23, 30). Recent publications have suggested that assays from different manufacturers recognize complexed and free cTnI forms differently (24, 30). The AccuTnI assay recognizes the binary cTnIC or cTnIT or ternary cTnICT complexes and free cTnI equally as deduced by reconstitution experiments using purified intact cTnI, cTnIT, and cTnC. The difference in antibody affinity to in vivo-modified or -truncated TnI complexes reported by Labugger et al. (22) remains to be investigated pending future characterization. The diagnostic and prognostic values of the spectrum of in vivo-modified troponin products in clinical practice have yet to be established.

The NACB has recommended the use of plasma as the preferred sample type because it allows more rapid analysis than serum (2). Matched-sample studies suggest that serum and heparin-plasma samples are acceptable but should not be used interchangeably (3). The use of heparinized plasma samples in some commercial assays may lead to lower recoveries for cTnI and cTnT than would be obtained with serum because heparin may bind directly to the troponin molecules and block the antibody epitope or may cause conformational changes in the molecules (31–33). It has also been shown that the use of EDTA plasma may affect cTnI assay results (34). The mechanism for the –14% average bias for EDTA-plasma results is not completely understood. EDTA may affect the alkaline phosphatase enzyme conjugate directly or change the stability of the cTnI molecule. EDTA plasma may be acceptable to aid in the diagnosis of MI and cardiac muscle damage. Different decision cutoffs may be required for this sample type. The absence of cross-reactivity in the AccuTnI assay to other myofibrillar proteins demonstrates its high specificity for cTnI. Analytical interference from circulating anti-animal antibodies, especially HAMAs, heterophilic antibodies, or RFs, has been a serious problem in many commercial two-site sandwich cTnI immunoassays (35). This incidence was very low in the AccuTnI assay.

Low abnormal troponin values within the indeterminate zone (i.e., between the URL and AMI cutoff points) have been proposed to be indicative of myocardial injury in the clinical setting of ischemic MI (2, 3). The 97.5 percentile URL, recommended by the NACB, has been replaced by the more recent ESC/ACC expert committee consensus proposal that an increased value for cardiac troponin be defined as a measurement exceeding the 99 percentile of a reference control group, using an assay with a CV <10% at this value. Two recent editorials provided further clarification (36, 37). To date, no troponin assays appear to achieve this target (13, 37). The functional sensitivity of the AccuTnI assay corresponding to an imprecision (CV) of 10% ranged from 0.05 to 0.11 μg/L (median, 0.06), which exceeds the 99 percentile of reference controls at 0.04 μg/L. In the current study, none of the 254 control individuals had a cTnI value ≥0.06 μg/L. In a separate clinical study, 62 of 68 (91%) non-AMI patients that had Access AccuTnI values above the 99 percentile URL (0.04 μg/L) were found to have cardiac...
conditions, such as unstable angina, congestive heart failure, and myocarditis, or severe noncardiac conditions, such as trauma or renal failure (14).

In conclusion, the Access AccuTnI assay system provides sensitive, precise determination of cTnI. Serum and heparin-plasma samples are acceptable. EDTA-plasma samples exhibited an average ~14% bias vs matched LiHep-plasma samples. The AccuTnI assay demonstrates minimal cross-reactivities and analytical interferences and excellent low-end precision. Method comparison data confirmed the heterogeneity in current commercial assays manufactured with different standardization, antibody pairs, and reagent formulations and reflected the expected differences in troponin values, specificity, stability, and analytical interferences. Nevertheless, strong agreements in classification of samples (as above or below respective cutoffs) were observed between AccuTnI and other commercial methods.

This study was funded by Beckman Coulter, Inc. We thank Judith Gray, Jennifer Gray, and James Carr (John T. Mather Memorial Hospital, Port Jefferson, NY); Stacy J. Wieczorek and Kathryn R. Baill (Hartford Hospital, Hartford, CT); Mary Ann Murakami, Heidi Quest, and Wendy Mathews (Hennepin County Medical Center, Minneapolis, MN); Ing-Britt Persson and Kerstin Lindblad (University of Uppsala, Uppsala, Sweden); and Jeff Todtleben, Susan Smith, Matthew Page, Yongyi Yu, and Robert Parson (Beckman Coulter, Inc., Fullerton, CA) for help in this study. Beckman Coulter, in collaboration with the principal investigators, developed the study design, collated and analyzed the multicenter data, and prepared this manuscript.

References


