Clinical Performance of the EMIT Vancomycin Assay
Kiang-Teck Yeo,1,2 William Traverse,1 and Gary L. Horowitz1,2

In evaluating the EMIT™ system for vancomycin used in the Cobas Bio™ centrifugal analyzer, we found two potential problems, each of which may have important clinical ramifications. First, precision, though acceptable in concentrations up to 30 mg/L, was marginal in the range above 30 mg/L. Second, when EMIT values were compared with those by fluorescent polarization immunoassay (TDx), we found a good correlation but a significant proportional bias: \[ \text{[EMIT]} = (0.877)[\text{TDx}] + 0.435 \text{ mg/L} \ (r = 0.971). \] The proportional bias was much more pronounced in specimens with high creatinine values than in specimens with normal values for creatinine. It remains to be determined which method is more nearly accurate. This imprecision and proportional bias in specimens with increased creatinines lead us to conclude that the EMIT vancomycin system should be used with caution.

Vancomycin is a glycopeptide antibiotic used in the treatment of sepsis caused by methicillin-resistant staphylococi (1-4). In addition, vancomycin is used in patients who are allergic to penicillins and cephalosporins, and in infections that cannot be treated with other drugs (1, 4). Because the incidence of such infections has increased, the use of vancomycin has increased concomitantly (5).

In infections due to susceptible organisms, therapeutic concentrations in serum (peak 30-40 mg/L) can be achieved with little risk of toxicity (1, 6). However, because of significant inter-individual variations in vancomycin pharmacokinetics, therapeutic drug monitoring is important to ensure efficacy (3, 7-11).

Current methods of measuring vancomycin in serum include bioassay (12), HPLC (13), RIA (14), and fluorescence polarization immunoassay (FPIA) (6). Recently, a homogeneous enzyme immunoassay (EMIT) for vancomycin (15) was introduced.

In this study we evaluated in detail the performance of the EMIT assay and compared results with those from the widely used FPIA system.

Materials and Methods

Reagents. Investigational vancomycin EMIT kits were provided by Syva Co. (Palo Alto, CA 94303). Reagent A contains mouse monoclonal antibodies to vancomycin, glucose 6-phosphate (G-6-P), nicotinamide adenine dinucleotide (NAD\(^+\)), and Tris buffer. Reagent B contains vancomycin labeled with glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and Tris buffer. A third component, EMIT assay buffer concentrate, contains Tris buffer, surfactant, and sodium azide (7.5 g/L). Six vancomycin serum calibrators (0, 5, 10, 20, 30, and 50 mg/L) are supplied to standardize the assay.

Instrument. We performed EMIT assays in a Cobas Bio centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ 07110), using the settings shown in Table 1. Data reduction was performed by the Cobas Bio with use of the "DENS" program for nonlinear standard curves provided by the manufacturer.

Samples. Samples routinely submitted to the laboratory for vancomycin assay were used for the comparison studies. Blood was collected in red-top Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070). After clotting for 10 min, the specimens were centrifuged and the sera were aliquoted. Most sera were analyzed immediately or stored at 4 °C if analysis could not be performed until the next day. However, to obtain a range of vancomycin concentrations, we also included some previously frozen sera in the study. Samples whose values exceeded the EMIT dynamic range were re-assayed after dilution in 0.11 mol/L NaCl. All EMIT results were compared with those by FPIA, done with TDx reagents in a TDx analyzer (Abbott Laboratories, North Chicago, IL 60064).

Procedure. The operating protocol for the EMIT vancomycin assay was that described by the Syva Co. (15). Reagents, calibrators, and controls were reconstituted with

<table>
<thead>
<tr>
<th>Table 1. Cobas Bio Settings</th>
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<tbody>
<tr>
<td>Units</td>
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<tr>
<td>Calculation factor</td>
</tr>
<tr>
<td>Standard 1 concn</td>
</tr>
<tr>
<td>Standard 2 concn</td>
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<tr>
<td>Standard 3 concn</td>
</tr>
<tr>
<td>Standard 4 concn</td>
</tr>
<tr>
<td>Standard 5 concn</td>
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<tr>
<td>Standard 6 concn</td>
</tr>
<tr>
<td>Limit</td>
</tr>
<tr>
<td>Temp. (°C)</td>
</tr>
<tr>
<td>Type of analysis</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sample volume (µL)</td>
</tr>
<tr>
<td>Diluent volume (µL)</td>
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<tr>
<td>Reagent volume (µL)</td>
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<tr>
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<td>Start reagent volume (µL)</td>
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<tr>
<td>Time of the first reading (s)</td>
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<tr>
<td>Time interval (s)</td>
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<tr>
<td>Number of readings</td>
</tr>
<tr>
<td>Blanking mode</td>
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<tr>
<td>Printout mode</td>
</tr>
</tbody>
</table>

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Results

Calibration curve. The calibration curve is constructed by plotting the enzyme activity of each standard minus the enzyme activity of the zero standard ("delta rate") vs the concentration of the standards. The delta rates generated with the five non-zero vancomycin calibrators were obtained for 10 between-run determinations of vancomycin. As shown in Figure 1 and Table 2, the change in delta rates decreases with increasing vancomycin concentration. Between the 30 and 50 mg/L calibrators, the imprecision in the response (delta rates) of the calibrators themselves (2 SD of approximately 10 mA/min) translates to imprecision in the dose of about 7.1 mg/L.

Precision. Precision was determined with the controls supplied by the manufacturer. The within-run CV was 7.2% for Level 1 (n = 20, x̄ = 6.9, SD = 0.5) and 5.7% for Level 2 (n = 20, x̄ = 40.3, SD = 2.3). The between-run CV was 8.5% for Level 1 (n = 29, x̄ = 7.7, SD = 0.7) and 6.1% for Level 2 (n = 29, x̄ = 38.1, SD = 2.3).

Analytical recovery. Using serum supplemented with vancomycin provided by the manufacturer (nine sets, spanning the range 5 to 50 mg/L), we determined vancomycin in duplicate by both EMIT and FPIA. Expressed as a percentage of FPIA, the average EMIT recovery was 95%.

Interference. Hemoglobin, lipid, and bilirubin were added to three different pooled specimens of human serum, which were then assayed to assess the effects of these common interferents on the EMIT assay. To avoid matrix effects, the interferents did not constitute more than 50 mL per liter of the total mixture. At 12 g of hemoglobin, 43 g of triglyceride, and 0.3 g of bilirubin per liter, no clinically significant interferences were observed.

Method comparison. Patients' samples assayed for vancomycin concurrently by EMIT and FPIA showed good correlation (r = 0.971), but a significant proportional bias (slope = 0.877, P < 0.001) (Figure 2A). To investigate this proportional bias in more detail, we subdivided the specimens into two groups on the basis of their creatinine concentration. For specimens with creatinine ≥16 mg/L (Figure 2B), the proportional bias was even larger (slope = 0.804, P < 0.001). In contrast, for specimens with creatinine <16 mg/L (Figure 2C), the proportional bias was significantly smaller (slope = 0.986, P < 0.001). The proportional bias between EMIT and FPIA persisted when fresh (n = 84) and frozen (n = 27) specimens were analyzed independently.

Stored calibration curve. We next studied the possibility of using a stored calibration curve for determination of vancomycin by EMIT. A computer program was written that simulated the Cobas Bio DENS software; its performance was verified by using it to calculate results for other EMIT methods with the same mathematical model. Rates obtained for the vancomycin calibrators on the first day were entered into the program to establish a "stored curve." The rates obtained for the calibrators and controls on subsequent days were entered into the program as unknowns, and the program calculated the corresponding vancomycin values in two different ways, with and without correction for the change in the enzyme activity of the zero calibrator. Based on 11 runs over 36 days, for each calibrator (except

<table>
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<th>Table 2. EMIT Calibration Curve</th>
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<tr>
<td>Investigational lot</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Calibrator concn, mg/L</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>50</td>
</tr>
</tbody>
</table>

* Defined as the change in delta rate between successive calibrators.

Fig. 1. Dose–response curve for EMIT vancomycin
Delta rate is defined as the difference in rate between calibrator, and calibrator, for the investigational kit (O) and for the production kit (O). The error bars denote ±2 SD from the mean (n = 10)

Fig. 2. Correlation between EMIT and TDx
(A) all specimens; (B) specimens with creatinine ≥16 mg/L; (C) specimens with creatinine <16 mg/L
perhaps 50 mg/L), analytical recoveries by either stored-
curve method appeared to be an acceptable alternative to
recalibration with each run (Figure 3). However, the
Levey–Jennings plots for the controls (Figure 4) indicate
that the values from the stored curve without zero-corrек-
tion were not as reproducible as either the values from the
stored curve with zero-correction or the values obtained on
running a new curve with each run. Thus it is valid to run
the EMT vancomycin assay for at least a month with use of
a stored curve protocol with zero correction.

Discussion
Our evaluation of the EMT assay for vancomycin on the
Cobas Bio provides some interesting dilemmas. As reflected
in the initial quality-control data collected, the EMT assay
exhibits good precision, similar to that of FPIA, HPLC, or RIA
(16, 17). Our comparison studies indicate that results for
patients' specimens correlate well with those by the widely
used TDx system. In addition, the EMT assay can be run
against a stored curve, thus minimizing costs. Nonetheless,
we discovered two potentially important problems.

Despite the good precision reflected in the quality-control
materials, the relative flatness of the standard curve be-
tween the 30 and 50 mg/L calibrators (Figure 1) prompted
us to do a dilution experiment. We chose two patients' spec-
imens, one of which approximated the Level 2 control
material and the other of which slightly exceeded the
highest calibrator. We assayed each specimen, undiluted
and twofold diluted, in 20 separate runs. As noted in Table
3, sample A had a mean value of 36.4 mg/L (undiluted) and
40.6 mg/L (diluted), and the precision associated with each
of these was comparable to that for the Level 2 control.
In contrast, sample B had a mean value of 44.6
mg/L (undiluted) and 56.9 mg/L (diluted). Of most concern
is the fact that sample B was not flagged as >50 mg/L in 14
of the 20 runs. Thus, a patient's specimen with a poten-
tially toxic concentration (57 mg/L) was assayed 70% of the
time as <50 mg/L. The therapeutic peak range of vanco-
mycin is commonly cited as 30–40 mg/L and toxicity is
associated with concentrations >40 mg/L (1, 4, 6, 10), so
this limitation of the EMT assay could lead to incorrect

therapeutic decisions.

Since our evaluation of the investigational lot, Syva has
released a production lot whose standard curve has im-
proved separation in the upper range (Figure 1, Table 2).
To assess in more detail the assay's precision in this area,
we assayed by EMT a series of specimens with various
vancomycin concentrations, specimens we prepared by sup-
plementing drug-free serum with a pharmaceutical prep-
paration of vancomycin. As shown in Table 4, despite good
precision of the commercial quality-control material, the
precision of the supplemented specimens, though accept-
able up to about 35 mg/L, became problematic at higher

![Fig. 3. Calibrator stability
The Cobas Bio data-reduction method was applied to the curve generated
from the calibrators run on day 1 to obtain the results "with zero-correction"
and "without zero-correction". Rates for the samples are corrected by using
the rate for the zero-calibrator either run at the same time [with zero-correction
() or run on day 1 [without zero-correction (○)]. Assigned calibrator values
are indicated by the dotted lines.](image-url)

![Fig. 4. Levey–Jennings plot of quality-control materials for vancomy-
cin by EMT
The dotted lines indicate the mean value for each control run with a new curve;
the solid lines indicate the corresponding 2 SD boundaries. Individual control
values are shown for each of three protocols: new curve (○), with zero-
correction (△), and without zero-correction (□). In general, all three methods
of data reduction are equivalent, except that from days 30 to 36 all the (△) are
below the −2 SD line.](image-url)

![Table 3. Sample-Dilution Experiment (Investigational
Kit)
<table>
<thead>
<tr>
<th>Twofold dilution</th>
<th>Undiluted</th>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
</tr>
<tr>
<td>A</td>
<td>40.6</td>
</tr>
<tr>
<td>B</td>
<td>56.9</td>
</tr>
</tbody>
</table>
* Two patients' samples (A and B) were assayed, undiluted and after two-
fold dilution, in 20 separate EMT runs. For B, only six of 20 determinations were
correctly flagged as >50 mg/L. Lowest value obtained for B: 39 mg/L.

![Table 4. EMT Precision Profile (Production Lot)
<table>
<thead>
<tr>
<th>Sample</th>
<th><strong>Mean</strong></th>
<th><strong>SD</strong></th>
<th><strong>CV, %</strong></th>
<th><strong>Minimum</strong></th>
<th><strong>Maximum</strong></th>
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<tbody>
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<td>0.9</td>
<td>3.8</td>
<td>22.5</td>
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<tr>
<td>A</td>
<td>10</td>
<td>31.9</td>
<td>2.2</td>
<td>6.9</td>
<td>28.3</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>35.3</td>
<td>2.5</td>
<td>7.1</td>
<td>31.0</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>38.8</td>
<td>3.5</td>
<td>9.0</td>
<td>31.4</td>
</tr>
<tr>
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<td>10</td>
<td>40.6</td>
<td>3.2</td>
<td>7.9</td>
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</tr>
<tr>
<td>E</td>
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<td>3.6</td>
<td>8.6</td>
<td>35.8</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>46.5</td>
<td>2.3</td>
<td>4.9</td>
<td>42.0</td>
</tr>
<tr>
<td>G</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>45.6</td>
</tr>
</tbody>
</table>
* Flagged as >50.0 mg/L on four of 10 runs.
concentrations. In particular, sample E, whose mean value was 42.0 mg/L, was assayed as low as 35.8 and as high as 49.0. Although we believe that the precision in the range above 30 mg/L is problematic, the problem is not insurmountable. Indeed, it could be eliminated by further modification of the assay by the manufacturer, along the lines already reflected in the transition from investigational to production lot. As a general practice, we strongly encourage laboratories to routinely assess precision in the high range, by running a sufficiently high control and by carefully examining the standard curve. Even if the manufacturer does not modify the assay, however, one can employ another strategy to compensate for the problem: re-run all specimens whose values exceed 30 (or perhaps 40) mg/L after dilution. Obviously, if the proportion of specimens in this concentration range is large, this strategy could prove expensive and impractical.

Our second concern stems from the correlation studies. Our comparison of patients' results, despite the good correlation, indicates a significant proportional bias of about 12% between EMIT and FPIA. However, the recovery study with supplemented sets indicates that results by the two methods should be similar. When we subdivided our patients based on their creatinine values (Figure 2B, C), the bias was minimized in patients with normal renal function but was more pronounced in patients with compromised renal function. One possible explanation of these results is that the FPIA antibody, but not the EMIT antibody, cross-reacts with a compound that accumulates in the serum of patients with renal insufficiency.

Such a suggestion is not without precedent. A recent report by Morse et al. (18) showed that FPIA can overestimate vancomycin concentrations in patients who are undergoing peritoneal dialysis by 13% to 53% compared with HPLC. These authors suggested that the overestimation by FPIA was caused by the accumulation of vancomycin degradation products that cross-react with the FPIA antibody. Interestingly, previous comparison studies between FPIA and HPLC showed good correlation but varying proportional bias, including negative bias (19), no bias (16, 20), and positive bias (6, 21, 22).

Thus, the existence of the proportional bias between EMIT and FPIA presents two questions: whether compounds are formed in vivo that cross-react with either antibody, and, if present, whether such compounds are pharmacologically active. If such compounds exist and are pharmacologically active, then FPIA values may well be appropriate. Otherwise, at least in some subsets of patients where such cross-reacting, pharmacologically inactive compounds accumulate, EMIT values are probably more nearly accurate. Until these questions are definitively answered, it is important to keep them in mind, no matter which method one chooses to use.

We are grateful to Ms. Brenda Bentley for her expert technical assistance in this evaluation. We thank Syva Company for providing us with the EMIT vancomycin reagents for the study.

References