individual to interindividual variation (16). When the index is low, particularly when it is <0.6, the dispersion of values for any individual will span only a small part of the reference interval. Reference values will thus be of little use, in particular for deciding whether a significant change has occurred. Conversely, when the index is high, particularly when it is >1.4, values from a single individual will cover much of the entire distribution of the reference interval. In this context, reference values will be of significant value for clinical interpretation. For total CoQ10, the index of individuality is 12.2/29.0 = 0.42, thus, it is low.

In conclusion, we have derived data for the biological variation of CoQ10 together with reference values for the New Zealand population. The low index of individuality for CoQ10 indicates that individual CoQ10 concentrations are tightly distributed around a homeostatic setpoint and that significant changes can occur within the reference interval. From a statistical perspective, seven samples should be evaluated to ensure that the estimate of the homeostatic setpoint is within 10% of the true value, with 95% probability (15). From a clinical perspective, serial changes in CoQ10 should be evaluated against the RCV to allow for both biological variation and analytical imprecision.

We would like to acknowledge the Tertiary Education Commission and the Health Research Council of New Zealand for financial assistance, Associate Professor Christopher Frampton for statistical advice, Core Biochemistry for lipid analyses, and Endolab for collaboration in the reference interval studies.

**References**


**Table 1. The 95% interfractile reference intervals for all analytes, ratios, and subgroups meriting stratification.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>95% Interfractile Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CoQ10</td>
<td>205</td>
<td>0.46–1.78 μmol/L</td>
</tr>
<tr>
<td>Males†</td>
<td>90</td>
<td>0.45–2.05 μmol/L</td>
</tr>
<tr>
<td>Females†</td>
<td>115</td>
<td>0.46–1.71 μmol/L</td>
</tr>
<tr>
<td>Age 18–44 years</td>
<td>105</td>
<td>0.43–1.61 μmol/L</td>
</tr>
<tr>
<td>Age 45–83 years</td>
<td>100</td>
<td>0.57–1.95 μmol/L</td>
</tr>
<tr>
<td>LDL-C</td>
<td>205</td>
<td>1.50–4.98 mmol/L</td>
</tr>
<tr>
<td>Ratio of total CoQ10 to LDL-C</td>
<td>205</td>
<td>1.50–4.98 mmol/L</td>
</tr>
<tr>
<td>TC</td>
<td>205</td>
<td>3.57–8.40 mmol/L</td>
</tr>
<tr>
<td>Ratio of total CoQ10 to TC</td>
<td>205</td>
<td>1.50–4.98 mmol/L</td>
</tr>
<tr>
<td>Males</td>
<td>90</td>
<td>1.21–284 μmol/mol</td>
</tr>
<tr>
<td>Females</td>
<td>115</td>
<td>88–244 μmol/mol</td>
</tr>
</tbody>
</table>

† These subgroups are not statistically required to be stratified based on Harris and Boyd criteria (11), but they may be of interest.

**Application of Commercial Calibrators for the Analysis of Immunosuppressant Drugs in Whole Blood, Thomas M. Annesley (University of Michigan Health Systems, 1500 East Medical Center Dr., Ann Arbor, MI 48109-0054; e-mail annesley@umich.edu)**

Multiple immunosuppressant drugs are now available to prevent organ rejection. Because of their different modes of action, these drugs are often prescribed together as part of a multidrug protocol. This has created a challenge for clinical laboratories to analyze multiple immunosuppressant drugs in the same blood specimen. HPLC–mass spectrometry (HPLC-MS) or -tandem mass spectrometry (HPLC-MS/MS) (1–6) assays can measure all of the common drugs.

A practical issue with the use of HPLC-MS/MS is the necessity of preparing whole-blood calibrators containing cyclosporine, tacrolimus, and sirolimus. With the introduction of everolimus, yet another set of calibrators will be needed. As with any assay for which in-house calibrators are prepared, this added variable will contribute to the interlaboratory differences observed in proficiency testing programs. The availability of either standard reference materials (SRMs) or uniform calibrators for use
among different laboratories would help with this problem.

Recently, the Thermo Electron and Waters Corporations announced alliances to distribute Chromsystems products. Among the Chromsystems products are whole-blood calibrators and controls for cyclosporine, tacrolimus, and sirolimus, with everolimus to be added in future lots of material. The availability of multiconstituent whole-blood calibrators or controls would address the issues stated above, if validation of their performance was to be performed in actual laboratory use. With this in mind I undertook a set of experiments to answer several important questions: (a) Do the assigned concentrations in these whole-blood materials appear to be accurate? (b) When they are used as the calibrators, do the assays yield results for patient specimens that are similar to those obtained when validated in-house calibrators are used for the assays? (c) Do they yield expected results for separate commercial whole-blood controls and for proficiency testing samples? (d) Are there any apparent matrix effects associated with these materials that could affect the accuracy or reproducibility of results?

The cyclosporine for in-house whole-blood calibrators was obtained from Novartis, and sirolimus was from LC Laboratories. For tacrolimus, I used Abbott Tacrolimus II calibrators. For internal standards, the cyclosporin D was from Novartis, the 32-desmethoxyrapamycin was from Wyeth, and the ascomycin was from Sigma. Lyphochek whole-blood controls were purchased from Bio-Rad, and external proficiency testing specimens were obtained from the College of American Pathologists (CAP). The commercial calibrator and control materials were from Chromsystems. These consisted of a set containing a whole-blood blank, a single whole-blood calibrator, and four quality-control (QC) whole-blood samples covering the range of concentrations that would be expected in patient specimens. The target (assigned) concentrations for cyclosporine, tacrolimus, and sirolimus have been verified for the company by an independent set of reference laboratories. Assigned concentrations are provided for immunoassay (cyclosporine and tacrolimus), HPLC with ultraviolet detection (cyclosporine), and HPLC-MS (sirolimus and tacrolimus). The assigned concentrations used for comparison of results were the HPLC-ultraviolet detection (cyclosporine) and HPLC-MS/MS (sirolimus and tacrolimus) values provided by Chromsystems.

HPLC-MS/MS analyses were performed with the instrumentation, chromatographic conditions, and multiple-reaction monitoring transitions monitored for the immunosuppressants described previously (7). Extraction of whole-blood calibrators, controls, proficiency samples, and human specimens was performed according to a recently published protocol (7). Because matrix effects or ion suppression could contribute to the performance of the commercial calibrators or controls (8), additional solid-phase extraction (SPE) using a 25-mg (1-mL) Varian LMS crossed-linked styrene-divinylbenzene column (7) was performed.

For the "in-house" calibrators, I prepared 6 and 30 μg/L whole-blood calibrators for sirolimus and 100, 200 and 400 μg/L whole-blood calibrators for cyclosporine, and used the Abbott 6 and 30 μg/L calibrators for tacrolimus. These calibrators and the Chromsystems whole-blood calibrator and controls were extracted and analyzed within a single run to avoid any day-to-day variation in instrument response. The in-house and Chromsystems samples were analyzed in three separate runs, and the mean measured concentrations were determined. Also included in runs were unidentified patient specimens, Bio-Rad Lyphochek controls, or CAP proficiency testing samples.

Using the in-house calibrators in my laboratory’s assay, I quantified the immunosuppressants in the Chromsystems calibrator and controls to see how closely the assigned values agreed with those measured. I then used the Chromsystems one-point calibrator (plus the zero blank sample) as the calibrator and measured the in-house calibrators and Chromsystems controls. Lastly, I used the four Chromsystems controls as the calibrators and measured the immunosuppressant concentrations in the other two materials. I also measured the concentrations of the immunosuppressant drugs in the Bio-Rad controls and CAP survey samples, using the in-house calibrators, the Chromsystems one-point calibration, or a four-point calibration using the Chromsystems controls as assigned calibrators.

The mean values for sirolimus calculated with use of the Chromsystems materials, the Bio-Rad controls, CAP proficiency samples CS-01 and CS-02, and the in-house calibrators are shown in Table 1. In the case of sirolimus, some differences >10% were noted (bold font in Table 1) when the calculated concentrations of sirolimus were compared for the Chromsystems materials and in-house whole-blood calibrators. This occurred for specimens prepared by zinc sulfate/methanol protein precipitation, but was not evident when the more extensive SPE preparation was performed. These results point toward a potential matrix effect (7, 8) rather than differences in the accuracy of the concentration assignments for the calibrators. This is also supported by the fact that the agreement of results for the CAP specimens and Bio-Rad controls was improved after the SPE cleanup.

For tacrolimus (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue2/), use of the Chromsystems materials or in-house whole-blood calibrators yielded acceptable agreement of results, whether the simple protein precipitation or the more complete SPE protocol was used for specimen preparation. Using any of the calibrator sets, I found acceptable results for the CAP samples.

For cyclosporine, the performance of the Chromsystems or in-house calibrator sets was also comparable (Table 2 in the online Data Supplement). The calculated results for the CAP proficiency specimens and Bio-Rad controls were in slightly closer agreement with target values when SPE was added to further clean up the whole-blood specimens and to presumably remove additional constituents that
could cause matrix effects. One would have to perform a separate study with different “artificial” or lyophilized specimens to verify whether a significant matrix effect exists for different HPLC-MS/MS cyclosporine assays.

To determine whether the comparability of the Chromsystems and in-house calibrators found above was a reproducible phenomenon, I evaluated a new lot of the Chromsystems four QC bloods, using these in a four-point calibration as in the experiments described above. The quantitative results obtained for four CAP proficiency samples and Bio-Rad controls when the two lots of the Chromsystems QC materials were used as the calibrators are shown in Table 3 of the online Data Supplement. The two calibrator sets provided similar results for tacrolimus, cyclosporine, and sirolimus.

The sirolimus linear regression data for patient whole-blood specimens, with and without SPE cleanup, are shown in Fig. 1, in which the results obtained with the Chromsystems materials are compared with the in-house calibrators. The intercepts in Fig. 1 are nearly the same, but the slopes are closer to 1.0 after SPE cleanup; the regression data therefore indicated that the addition of SPE cleanup improved the agreement of results. Because the same patient samples were used for all experiments in Fig. 1, a paired t-test was performed to verify this observation (Table 4 in the online Data Supplement). The P values in supplemental Table 4 demonstrate that when the Chromsystems materials were compared with in-house calibrators, the differences in patient results for paired samples were statistically significant if the simple precipitation protocol was used but not significant if additional SPE cleanup was included. The results for patient specimens quantified by use of the in-house calibrators, with or without SPE, were not statistically different. This improvement in agreement for patient specimens after SPE purification also parallels the data in Table 1, in which the agreement for sirolimus in CAP surveys and Bio-Rad controls was also better after additional SPE cleanup.

For tacrolimus, Fig. 1 in the online Data Supplement shows the excellent correlation and agreement for patient results with all selected calibrator sets. All slopes were close to 1.0, and no intercept (background) effects were present whether simple protein precipitation or additional SPE cleanup was performed. The comparisons for cyclosporine (Fig. 2 in the online Data Supplement) showed statistically equivalent quantitative results (P = 0.187–0.704) for patient specimens when the Chromsystems material was used vs in-house calibrators. Because trough cyclosporine concentrations are routinely monitored at my institution, no random patient specimens with higher concentrations (e.g., c2 values) were selected; the study was therefore limited to specimens with concentrations ranging from 50 to 400 μg/L.

Several conclusions can be drawn from these data. The first conclusion is that the assignments for the immunosuppressant drugs for the Chromsystems materials agree with other in-house or commercial whole-blood calibrators for cyclosporine, tacrolimus, and sirolimus. The agreement extended to more than one lot of material. Comparable values were obtained for three types of samples: a commercial whole-blood control, CAP proficiency testing specimens, and actual human whole-blood specimens. In many laboratories, the regulatory agencies require multiple-point calibrations; the Chromsystems four QC materials can be used for this purpose. QC materials from other sources (e.g., Bio-Rad) can then be used.

The second conclusion is that although all of the calibrators tested performed acceptably with proficiency testing, QC, and patient samples, the agreement among values was slightly better when a more extensive SPE cleanup was used. This might be expected because previous work has shown, for these immunosuppressant drugs in whole blood, that ion suppression and assay imprecision are dependent on the type of specimen extraction protocol used before liquid chromatography–MS/MS analysis (7). Nonetheless, the Chromsystems calibrator materials do not appear to be associated with, or subject to, severe matrix effects when used with the water hemolysis precipitation method that was used in this study. Because these are lyophilized materials and may contain...
unspecified stabilizers, plasticizers, and other components, matrix effects could still be a possibility if other specimen preparation protocols are used.

References


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Cardiac Troponin and Creatine Kinase MB Monitoring during In-Hospital Myocardial Reinfarction, Fred S. Apple and MaryAnn M. Murakami (Department of Laboratory Medicine and Pathology, Hennepin County Medical Center and University of Minnesota School of Medicine, Minneapolis, MN; * address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories P4, 701 Park Ave., Minneapolis, MN 55415; fax 612-904-4229, e-mail fred.apple@co.hennepin.mn.us)

Cardiac troponin monitoring for detection of myocardial injury has been designated the new standard for differ-