We have characterized the CYP activities for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 from 50 donors. The mean enzyme activities are presented in Table 4 and range from 0.03 to 0.18 pmol/min/mg protein, with the highest observed activity for CYP2C19. These activities can be used to predict the expected variability in the CYP enzymes from a 50 donor pool. The table also shows the actual variability predicted from the Monte Carlo analysis, which is in good agreement with the observed variability. This information is useful for designing studies and estimating the number of donors required for a given level of confidence in the results.

Results (continued)
As discussed earlier, the distributions of CYP activities were skewed to higher activity levels and did not follow a normal distribution. We have used Monte Carlo analysis to predict the expected variability in random pools of differing sizes. Data are from 30 trials. For this exercise, CYP2C19 is pooled based on an equal mg protein basis. Figure 4 provides an illustration of the result for CYP2C19 for pools ranging from 25 donors to 200 donors. The distribution of calculated pooled CYP activities was found to be Normal and CVs were calculated from the mean and standard deviations. The calculated CV decreased from 0.27 to 0.07 and the donor number increased from 25 to 200. As is evident from the graph, the donor number increased significantly over 100 and the reduction in CV is relatively modest.

Table. Calculated CVs from Monte Carlo analyses for six CYP enzymes and HLM pools of 50, 100, and 200 donors.

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>50 Donors</th>
<th>100 Donors</th>
<th>150 Donors</th>
<th>200 Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>0.09</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>0.14</td>
<td>0.08</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.16</td>
<td>0.10</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>0.17</td>
<td>0.10</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.12</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.14</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Summary and Conclusions
1. We have analyzed CYP activity levels in a panel of over 300 human donor livers, and have concluded the expected high level of variability in CYP activity with CYP2B6 and CYP19 being the most variable and CYP2C9 being the least variable.
2. There was an over representation of male donors relative to female donors.
3. The expression of CYP2C9 was found to be statistically higher in males relative to males.
4. The expression of CYP19 was found to be higher in young donors.
5. Monte Carlo analysis was used to predict the variability (CV) in HLM pools of different sizes.
6. The validity of this approach is supported by a retrospective study with 50 donor pool.
7. The Monte Carlo analysis indicates that a donor number of 150 will substantially reduce variability in HLM pools relative to the 50 donor pools which are currently employed.
8. The total of average CYPs has lead BD Biosciences to design and manufacture BD UltraPool® HLM 150. The design is 150 donors with a 50:50 male/female split, equal mg protein from each donor and pediatric livers excluded. The results of 4 actual QC assays relative to the overall mean and predicted upper and lower confidence levels are presented in Figure 6. There is good agreement between observed and predicted values, especially given that product variability is expected to be less than assay variability.

References