Abstract

Background: The automated Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 (CAP/CTM) and Abbott RealTime HIV (RealTime) quantitative RT-PCR assays were recently FDA-approved for viral load testing. While the assays are generally concordant, some studies have shown significant differences in quantification for some samples. We studied a large number of samples in both assays to determine the frequency and severity of underquantification.

Methods: Plasma samples with sufficient volume remaining after CAP/CTM testing were deidentified, aliquoted, and stored at -20°C in conjunction with corresponding CAP/CTM results and demographic information. The results were analyzed by Bland-Altman analysis. A discrepant sample was defined when the difference between the assay results was more than 2 standard deviations from the mean or when one assay measured more than 1 log copies/mL above its limit of quantification (LOQ) and the other assay was below its LOQ. Discrepant samples were retested in both assays using plasma diluted fivefold with Basematrix (Seracare, Milford, MA).

Results: Of the 1356 valid results, 472 were quantitative in both assays, 772 were below the LOQ in both assays, 80 were quantitative in CAP/CTM but below the LOQ in RealTime, and 32 were quantitative in RealTime but below the LOQ in CAP/CTM. The mean log copies/mL difference (CAP/CTM – RealTime) was 0.18 with a mean ± 2 standard deviation range of -0.51 to 0.86. The most discrepant quantitatively different samples had differences of 1.85 and 1.50 log copies/mL. The largest difference between samples that was less than the LOQ in the other assay was 4.65 log copies/mL. Thirty-eight discrepant samples (2.8%) were identified and retested in both assays. Seventeen samples had repeated differences (corrected for dilution) of less than 2 SD. Seven samples were indeterminate. Fourteen samples had discrepant results that require further analysis.

Conclusions: Comparison of a large number of samples from all regions of the U.S. identified a small number of discrepant results. Relative underquantification of similar magnitude was seen for both assays. These data support the importance of considering retesting using an alternate method when the results do not correlate with the clinical scenario.

Results

Table 1: Correlation agreement. The 1356 samples which were originally tested in CAP/CTM and repeated in RealTime were segmented by result. For the purposes of this table, no distinction was made between “detected but not quantitated” and “not detected” samples. Quant = quantitative result obtained, DNQ = detected but not quantitated, ND = not detected.

<table>
<thead>
<tr>
<th>Category</th>
<th>RealTime</th>
<th>CAP/CTM</th>
<th>Difference</th>
<th>Correlation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quant</td>
<td>472</td>
<td>472</td>
<td>0</td>
<td>Sy/x = 0.96, R² = 0.92</td>
</tr>
<tr>
<td>DNQ + ND</td>
<td>32</td>
<td>772</td>
<td>-0.49</td>
<td>Sy/x = 0.342, R² = 0.923</td>
</tr>
</tbody>
</table>

Table 2: Discrepant sample details. The details for each of the 38 discrepant samples are shown.

<table>
<thead>
<tr>
<th>State</th>
<th>Original Result</th>
<th>Difference</th>
<th>Correlation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>2.9</td>
<td>1.9</td>
<td>Sy/x = 0.96, R² = 0.92</td>
</tr>
<tr>
<td>UT</td>
<td>2.5</td>
<td>2.8</td>
<td>Sy/x = 0.342, R² = 0.923</td>
</tr>
<tr>
<td>TN</td>
<td>2.7</td>
<td>2.7</td>
<td>Sy/x = 0.96, R² = 0.92</td>
</tr>
<tr>
<td>NE</td>
<td>5.9</td>
<td>ND</td>
<td>Sy/x = 0.342, R² = 0.923</td>
</tr>
</tbody>
</table>

Summary

- The overall correlation of the CAP/CTM and RealTime assays was excellent.
- The average difference (CAP/CTM - RealTime) among samples with quantitative results in both assays was 0.18 log copies/mL.
- Of 1356 valid samples received from 32 U.S. states, 38 (2.8%) were determined to be discrepant after the initial analysis.
- The discrepant samples were received from 18 different states.
- The most discrepant quantitative samples had differences of -1.85 and 1.50 log copies/mL, while the largest difference between samples that was less than the LOQ in the other assay was 4.65 log copies/mL.
- After retesting the 38 discrepant samples (fivefold dilutions) in both assays, 17 were no longer discrepant, 7 were indeterminate, and 14 remained discrepant.
- Relative underquantification of similar magnitude and frequency was seen for both assays.
- These data support the importance of considering retesting using an alternate method when the results do not correlate with the clinical scenario.

References

4) Gueldner et al. 2007. JAIDS 44:500-505.

Materials and Methods

Sample selection: Plasma samples received by ARUP Laboratories for CAP/CTM HIV-1 testing were analyzed. Patient samples with sufficient volume (>1.5 mL) remaining following CAP/CTM testing were collected, regardless of the CAP/CTM result. These samples and their associated CAP/CTM results were deidentified, aliquoted, and stored at -20°C. A 1 mL aliquot was retested using the 0.5 mL version of the RealTime HIV assay. The excess 0.5 mL following this testing was refrozen.

Data analysis: The results of RealTime testing were compared to CAP/CTM by correlation. Deming regression, and Bland-Altman analysis. For the purposes of visualizing potential discrepancies, samples with quantitative results in the RealTime assay but "not detected" or "detected below 1.7 log" in CAP/CTM were assigned a CAP/CTM result of "0". Samples with quantitative results in CAP/CTM but negative in RealTime were assigned a RealTime result of "0". Samples with quantitative results in CAP/CTM but "detected below 1.8 log" in CAP/CTM were assigned a result of "0". Samples with quantitative results in both assays were used in the Deming regression and Bland-Altman analysis.

Discrepant analysis: Two classes of samples were deemed to be discrepant: 1) samples with a difference between the two assays of two standard deviations (SD) or more from the mean, and 2) samples for which one assay result was below the limit of quantification (LOQ) and the other assay result was more than 1 log copies/mL, greater than its LOQ. Samples meeting these criteria were repeated by diluting the remaining 0.4 mL plasma from RealTime testing with Basematrix (Seracare, Milford, MA) fivefold and retesting in both CAP/CTM and RealTime assays. The retesting results were adjusted for the dilution factor and compared to the original results. Samples for which the difference between the repeat results were 2 SD or more from the mean were categorized as still discrepant. Samples which were "not detected" or had dilute detected errors were categorized as "indeterminate".

Acknowledgements

The authors wish to thank Denise Jones for deidentifying and aliquoting the samples, Lauren Vest and Jung (Jessica) Lee for performing the RealTime analysis, and Brian Mullen for their technical support. This study was performed using m2000 systems and reagents provided by Abbott Molecular.

Presentation and Contact Info

Abstract W-133 : Poster 942 : Session 190 Michael T. Pyne, M.S. 17th Conference on Retroviruses and Opportunistic Infections San Francisco, CA February 16-19, 2010 michael.pyne@aruplab.com 801-583-2787 x3431