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Reagents

High concentration HIV-1 standard at 1.5 x 10^6 copies/mL was kindly provided by the Virology Quality Assurance (VQA) Laboratory, Rush University, Chicago, IL (provided under the NIAID Virology Quality Assurance Contract [J. Bremer, HHSN2722002003C, HHSN266200500044C]). The following Abbott RealTime reagents were obtained from the manufacturer (Abbott Molecular, Des Plaines, IL): RealTime HIV-1 Controls (Part # 6L-18-80), RealTime Calibrators (Part #6L-18-70), mSystem RNA general purpose reagent (Part # 04J79-24), mSystem DNA GPR modified for total nucleic acid extraction (Part #06K12-24), Bulk Lysis Buffer (Part # 02N77-01) and RealTime HIV-1 amplification kit (Part # 6L-18-90). Sera Care Base Matrix BM53 serum (Sera Care Life Sciences, Milford, MA) was used for bone marrow plasma dilutions. This reagent has been validated for plasma dilution assays for viral load testing by the VQA, the AIDS Clinical Trials Network (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) Laboratory Committees.

Viral load testing

Pooled bone marrow plasma were spiked with known HIV-1 concentration (500,000 copies/ml) used for standard quality assurance. Dilutions from this concentration were made to achieve the range of HIV concentrations used in the study. The prepared bone marrow plasma concentrations were further diluted 5-fold at the time of assay in either Abbott lysis buffer or Sera Care base matrix to final HIV-1 nominal concentration. All assays were performed on the Abbott m2000sp and m2000rt platforms following the same algorithms used for peripheral blood testing. Assays were performed on the Abbott platforms using 0.6 mL input volumes (0.8 mL with void volume). RNA extraction reagents were used for sample extraction according to manufacturer’s procedures [5]. Extractions were performed on the m2000sp automated extraction platform with PCR reactions completed on the m2000rt real time PCR platform.

Results

Bone marrow preparation for extraction

Initially, Abbott Lysis Buffer was used to dilute the spiked bone marrow, but recovery of HIV-1 was noted to be less than expected particularly with lower nominal viral concentrations. As a result, the assays were repeated using SeraCare BM53 as the diluent. HIV-1 recovery with the Abbott lysis buffer as the diluent was about 10 fold less efficient than with Sera Care BM53. Many centrifuged bone marrow samples had a lipid layer on top and with an indistinct interface with the plasma that could not be removed without significant loss of harvested marrow plasma. We suspect that the lipid layer does not adequately disperse in the Abbott Lysis Buffer leading to extraction interference but does solubilize in BM53 resulting in better viral recovery. A similar assay was performed without bone marrow plasma by diluting HIV-1 VQA Standard directly in either Abbott Lysis buffer or BM53 to determine if the discrepancy in recovery was influenced by the diluent. While there were small differences in viral recovery, the differences did not explain the bone marrow/diluent differences (data not shown).

Linearity

Pooled bone marrow plasma were spiked with HIV-1 VQA standard and diluted with BM53 to desired nominal concentrations of 100,000, 1,000, 100, 50 and 20 copies/ml. Two independent assays with replicate testing of nominal concentrations varied with 2 or 3 replicates of (1,000, 500 and 250 copies/ml), 2 or 7 replicates of 100 copies/ml, 15 total replicates of 60 copies/ml and 20 total replicates of 40 and 20 copies/ml. Table 1 depicts the results of the LLoD assays. The data suggest that the Abbott assay can reliably detect HIV-1 viral load in bone marrow plasma down to 250 copies/mL and sometimes as few as 40 copies/mL.

Discussion

Our study shows that the Abbott RealTime HIV-1 assay has potential utility in quantifying HIV-1 viral load in bone marrow plasma despite some limitations related in part to differences in bone marrow composition relative to peripheral blood plasma. The delay in processing the samples in our study resulted in the pools we used. The limited availability of pediatric bone
marrow plasma will likely require routine dilutions of bone marrow plasma in the Abbott assay clinically and if so the commercial Abbott lysis buffer may not be well suited for bone marrow plasma viral load testing. Instead SeraCare Basematrix BM53 should be used as it was better suited for bone marrow plasma viral load determinations in our study.

Our quantitative bone marrow plasma viral load determinations with the Abbott RealTime HIV-1 assay had a higher than expected lower limit of detection. The normal lower limit of quantitation of this assay in peripheral blood plasma is 40 copies/mL as compared to 250 copies/mL for bone marrow plasma found in this study. In addition we could not determine linear ranges beyond 100,000 copies/mL. Higher viral load samples would need additional dilutions to fall within the assay linear range that we found. Further studies comparing the viral load ratio between the blood and bone marrow plasma are needed to verify the utility of our method. Additionally, bone marrow viral load may have applications in other viral infections such as hepatitis C virus.

In conclusion, our data suggest that the Abbott RealTime HIV-1 assay can be adapted by using BM53 serum diluent to accurately detect HIV-1 viral activity in human bone marrow plasma over a range of 250 to 100,000 copies/mL.

References

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5. Abbott RealTime HIV-1 RNA PCR Kit Product Insert.