



Optimization of Abbott m2000 RealTime HIV-1 Viral Load Assay on Breast Milk, Dried Blood Spots, Seminal Plasma, Cerebrospinal Fluid, Urine, and Cervical Swabs



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CORRECTED ABSTRACT

Background: The latest technology for measuring HIV-1 RNA in plasma is the real-time PCR platform. Abbott's m2000 RealTime HIV-1 assay is CE-marked and FDA-approved for plasma only, but has not been optimized for off-label use with other sample types. Measurement of HIV-1 RNA levels in other body fluids, including breastmilk (BMK), dried blood spots (DBS), seminal plasma, and cerebrospinal fluid (CSF), is important for understanding pathogenesis and transmission of HIV. More accurate and precise methods for measuring HIV RNA levels in these other sample types are needed.

Methods: Plasma, BMK, whole blood, semen, and CSF were obtained from uninfected donors. 1:10 dilutions of an HIV-1 virus stock was added to plasma, whole BMK, whole blood (for DBS), seminal plasma, and CSF to generate series of samples containing 0.6 log₁₀ copies of HIV-1 per ml of fluid. Each sample type was lysed in different volumes of lysis buffer, with BMK and semen treated with proteinase K prior to extraction. Different m2000sp protocols were used to accommodate different sample volumes after addition of lysis buffer. HIV-1 RNA levels were measured off-label using the RealTime HIV-1 viral load assay in the m2000rt after extraction and the data from the different sample types were compared with the results from the spiked plasma samples.

Results: The results from the dilution series in plasma, BMK, semen, CSF, and DBS had linear results compared to the nominal cp/ml. Comparison of the samples to plasma indicated that HIV-1 RNA recovery after adjusting for sample input and dilution was near 100% from semen, CSF, and DBS, and approximately 50% from BMK.

Conclusions: The Abbott m2000 RealTime HIV-1 assay works well for measuring HIV-1 RNA levels in BMK, semen, CSF, and DBS. More accurate and precise measurements are critical for understanding pathogenesis and transmission, and for clinical trial evaluations.

Additional data: We expanded our studies to include urine (URN) and cervical swab (CXS) samples. Samples of each type were collected from uninfected donors and spiked with our HIV-1 virus stock. Results with urine were similar to CSF, and results from CXS were similar to semen.

INTRODUCTION

HIV viral loads are typically performed on peripheral blood plasma, but other compartments such as the uro-genital tract, nervous system, breast and oral cavity, may be potential sanctuary sites harboring HIV and impacting both the transmission and pathogenesis of HIV infection. From a patient's perspective it is important to determine whether anti-retroviral treatment can reduce viral loads in non-blood compartments, which can serve as potential reservoirs for viral replication. And from a public health standpoint, it is critical to understand the factors that contribute to "infectiousness" of an individual in order to develop strategies to reduce the likelihood of transmission. Increased viral loads in seminal plasma, cervical fluid, breast milk, and possibly saliva, probably contribute to increased transmission risks.

In addition, detection of HIV viral nucleic acids in samples that are readily collected and do not require phlebotomy (dried blood spots, urine, or saliva) are becoming important for diagnosis of infants, acute HIV infection and HIV transmission among vaccine recipients – all cases where the detection of HIV antibodies are not useful for diagnosis.

Many papers have been written in the last 15 years on the use of various HIV RNA assays (Roche Amplicor, NASBA, NucliSens, etc.) with different specimen types. Recently new real-time PCR platforms have been introduced (Abbott m2000 RealTime and Roche's Taqman). The m2000 RealTime HIV-1 assay is CE-marked and FDA-approved for plasma only, but has not been optimized for off-label use with other sample types. We evaluated the performance characteristics of the m2000 RealTime assay using seminal plasma, cervical swabs, urine, CSF, breast milk, and dried blood spots (DBS).

METHODS

- Sample types other than plasma were tested for viral load using the Abbott RealTime HIV-1 RNA Assay.
- Changes in sample preparation from the plasma protocol were tested using spiked samples collected from HIV-uninfected donors.
- Lysis buffer was obtained from Promega (catalog number Z3051) and Proteinase K from Abbott.
- A high titered HIV-1 stock was diluted in each sample type to test linearity and limit of detection.
- Panels of 10-fold dilutions were made to test each sample type in triplicate.
- All sample types run using 0.6 ml plasma protocol on m2000sp and m2000rt.

Sample type	Pre-treatment Protocol
BMK	1) 0.6 ml whole or skim BMK + 209 ul Lysis buffer + 60 ul Prot. K 2) 53°C 20 min
DBS	1) Cut out 2 whole 50 ul blood spots, add 1.7 ml Lysis buffer 2) Rock at ambient temperature 2 hrs 3) Remove 0.8 ml eluate for run
SEM	1) 0.4 ml seminal plasma + 0.4 ml Lysis buffer + 40ul Prot K 2) 53°C 20 min
CSF	Run as plasma
URN	Run as plasma
CXS	Run as plasma (samples had been collected in NASBA lysis buffer from bioMerieux)

RESULTS

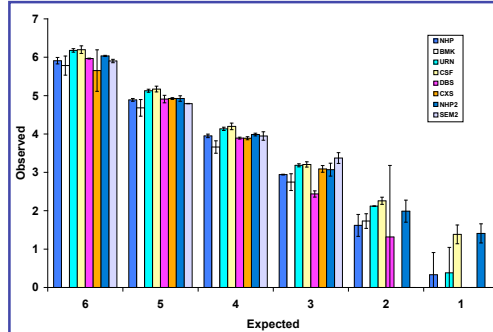


Figure 1. Results of triplicate testing of spiked samples with the Abbott RealTime HIV-1 RNA Assay. The average log cp/ml for each sample type is plotted with standard deviation. Semen samples were spiked along with NHP in a separate panel from the other sample types, so NHP is shown twice.

Table 1. Detectable HIV-1 RNA results for each sample type at the low end of the dilution series, indicating sensitivity (10³-10¹) and specificity (Negative) for each sample type.

	10 ³	10 ²	10 ¹	Negative
Plasma	6 of 6	6 of 6	5 of 6	0 of 6
Breast milk	3 of 3	3 of 3	0 of 3	0 of 3
Urine	3 of 3	3 of 3	1 of 3	0 of 3
CSF	3 of 3	3 of 3	3 of 3	0 of 3
DBS	3 of 3	1 of 3	0 of 3	0 of 3
Cervical swab	3 of 3	0 of 3	0 of 3	0 of 3
Semen	3 of 3	0 of 3	0 of 3	0 of 3

Table 2. Limit of detection and specificity for each sample type.

	Limit of detection	Specificity
Plasma	10 ¹	100%
Breast milk	10 ²	100%
Urine	10 ¹ -10 ²	100%
CSF	10 ¹	100%
DBS	10 ² -10 ³	100%
Cervical swab	10 ³	100%
Semen	10 ³	100%

CONCLUSIONS

- The Abbott RealTime HIV-1 RNA Assay can be used to measure HIV-1 RNA levels in all sample types tested here.
- Pre-treatment of the sample types varies, but all samples can be tested using the 0.6 ml plasma protocol.
- Specificity was 100% for all sample types.
- The lower limit of detection was best for plasma, CSF, and urine, and worst for genital secretions.

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