

Evaluation of an Automated Sample Preparation System and Real-Time Assay for Quantitation of Genetically Diverse HIV-1 Specimens from Brazil

*J.Hackett, Jr.¹, P. Swanson¹, V. Holzmayer¹, J. Yamaguchi¹, S. Huang², P. Bodelle¹, C. Brennan¹, R. Badaro^{3,4}, C. Brites³, and S.G. Devare¹

R. Hackett Jr.
Dept. 09NG, Bldg. AP20
Abbott Laboratories
100 Abbott Park Road
Abbott Park, Illinois 60064-6015
Tel: (847) 938-0457
Fax: (847) 937-1401
e-mail: john.hackett@abbott.com

#665

¹Abbott Laboratories, Abbott Park, IL, USA ²Abbott Molecular, Inc., Des Plaines, IL, USA ³Fundação Bahiana de Infectologia, Universidade Federal da Bahia, Bahia, Brazil ⁴University of California, San Diego, CA, USA

ABSTRACT

Background: The combination of automated sample preparation and real-time RT-PCR for measurement of HIV-1 viral load has the potential to significantly enhance throughput, reduce operator-associated error, and improve assay sensitivity and dynamic range. Due to the high rate of evolution and ever-changing distribution of HIV-1, reliable patient monitoring requires that viral load assays detect and accurately quantify genetically diverse strains of HIV-1. The aim of this study was to evaluate performance of the Abbott RealTime™ HIV-1 assay with the m2000sp automated sample preparation instrument (Abbott Molecular, Inc., Des Plaines, IL; not available in the U.S.) on a panel of genetically diverse specimens.

Methods: The specimen panel consisted of 91 HIV-1 seropositive plasmas collected from blood donors in Brazil. Subtype was determined by sequence/phylogenetic analysis of three independent genomic regions: gag p24, pol integrase, and env gp41. Specimens for which subtype assignments differed between the regions analyzed were categorized as mosaics. Viral loads were measured in the Abbott RealTime HIV-1 assay (m2000sp sample preparation and m2000rt amplification and detection instruments) and LCx[®] HIV RNA Quantitative assay (LCx HIV; Abbott Laboratories, Abbott Park, IL; not available in the U.S.).

Results: Group/subtype was determined for 89 of 91 specimens. The panel included 89 subtype B, 1 C, 2 F, and 17 mosaic strains. Eighty-nine specimens were quantified by the RealTime HIV-1 assay, 87 by the LCx HIV assay. The observed correlation for 86 specimens within the dynamic range of both assays was 0.922 with a slope of 0.853 and intercept of 0.772. Two specimens below the LLD of the LCx HIV assay (50 copies/mL) were quantified in the RealTime HIV-1 assay. Notably, two specimens below the LLD of both assays were also PCR-negative for all three regions used for subtype analysis. Good agreement was observed between assays with 90% of values within 0.5 log₁₀ copies/mL.

Conclusions: In the present study, the automated RealTime HIV-1 assay and sample preparation system accurately quantified this genetically diverse panel. Viral load determinations were highly correlated with the LCx HIV assay. The automated RealTime HIV-1 assay offers the advantages of increased throughput and reduced labor while providing reliable quantification of diverse HIV strains.

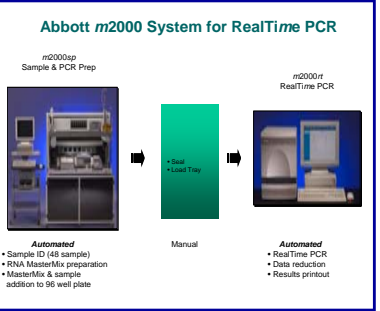
INTRODUCTION

Nucleic acid extraction is the most time-consuming and challenging aspect of viral load measurement. Manual methods require skilled technical personnel and extended "hands-on" time. Automation of the extraction process has the potential to significantly increase reliability, sample throughput and efficiency.

Application of real-time RT-PCR for HIV-1 quantitation can improve sensitivity, extend dynamic range and reduce risk of contamination.

Abbott automated m2000sp and m2000rt instruments were developed to improve molecular diagnostic test performance, throughput and reliability. The Abbott RealTime HIV-1 assay was designed for robust and accurate quantification of genetically diverse HIV-1 strains. In this study, we evaluate Abbott's automated instrumentation and RealTime HIV-1 assay on specimens from Brazil.

INSTRUMENTS



METHODS

- Collect HIV seropositive blood donations from Brazil
 - Characterize HIV-1 subtype of each strain
 - Nucleic Acid Extraction
 - RT-PCR (gag p24, pol integrase, env gp41 IDR)
 - Direct Sequencing
 - Phylogenetic Analysis
 - Perform Viral Load Testing
 - Abbott LCx HIV RNA Quantitative Assay*
 - Abbott RealTime HIV-1 Assay*
 - Evaluate nucleotide mismatches at primer/probe binding sites
- * not available in the United States

Viral Load Test Characteristics

Target	LCx HIV RNA Quantitative (LCx)	RealTime HIV-1 (RealTime)
pol integrase	pol integrase	pol integrase
LLQ (copies/mL)	50 copies (1ml sample)	40 copies (1ml sample)
ULQ (log ₁₀ copies/mL)	6 log ₁₀	7 log ₁₀
Sample Prep	Qiagen or m1000	m1000 or m2000sp
Plasma Volume	0.2 or 1 mL	0.2, 0.5 or 1 mL
PCR	Competitive PCR	Non-competitive PCR
Measurement	Endpoint (LCx MEIA)	RealTime (m2000rt)
Detection	Chemiluminescence	Fluorescence

RESULTS

Fig 1. Panel Composition (n = 91)

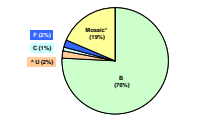


Fig 2. Range of Viral Loads

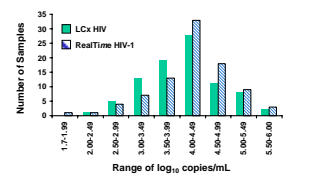


Fig 3. Viral Load Performance (n = 91)

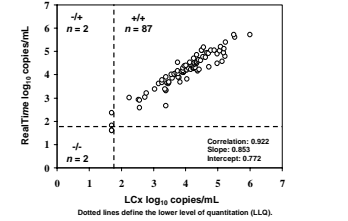


Fig 4. Agreement Between Methods (Bland Altman)

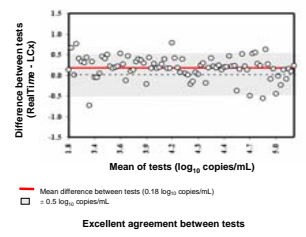


Table 1. Primer/Probe Nucleotide (nt) Mismatch Analysis

	HIV-1 Group M Subtype (n)				
	B (74)	C (1)	F (4)	BF (5)	Overall
LCx					
Forward	0.01 (0.00 - 0.02)	0.02 (0.02)	0.03 (0.03)	0.01 (0.02 - 0.03)	0.01 (0.00 - 0.03)
Probe	0.01 (0.00 - 0.11)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.01 (0.00 - 0.11)
Reverse	0.02 (0.00 - 0.05)	0.05 (0.05)	0.03 (0.00 - 0.05)	0.03 (0.00 - 0.05)	0.02 (0.00 - 0.06)
Total	0.01 (0.00 - 0.04)	0.03 (0.03)	0.03 (0.01 - 0.03)	0.01 (0.00 - 0.01)	0.01 (0.00 - 0.04)
RealTime					
Forward	0.01 (0.00 - 0.03)	0.03 (0.03)	0.03 (0.03)	0.01 (0.00 - 0.03)	0.01 (0.00 - 0.03)
Probe	0.05 (0.02 - 0.13)	0.02 (0.02)	0.03 (0.02 - 0.05)	0.04 (0.02 - 0.05)	0.05 (0.02 - 0.12)
Reverse	0.02 (0.00 - 0.13)	0.04 (0.04)	0.03 (0.00 - 0.04)	0.02 (0.00 - 0.04)	0.02 (0.00 - 0.13)
Total	0.03 (0.01 - 0.05)	0.03 (0.03)	0.03 (0.03 - 0.04)	0.02 (0.02 - 0.03)	0.03 (0.01 - 0.05)

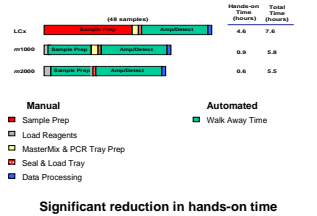
* mean (range) = no. of nt mismatches per assay
n = no. of primer or probe sites

Comparison of Primer/Probe Length

	Forward Primer	Probe	Reverse Primer
LCx	20 nt	20 nt	20 nt
RealTime	20 nt	20 nt	20 nt

High degree of nucleotide conservation at primer & probe sites for LCx and RealTime HIV-1 assays

Fig 5. The Automation Advantage



CONCLUSIONS

- Performance of the Abbott RealTime HIV-1 assay on this genetically diverse panel of Brazilian plasma samples was highly correlated to the LCx HIV RNA Quantitative assay; 90% of the samples (77/86 within the dynamic range of both tests) had values within 0.5 log₁₀ copies/mL.
- Two specimens with low viral loads (68 & 229 copies/mL) were only quantified by the RealTime HIV-1 assay.
- Similar to the LCx HIV assay, the RealTime HIV-1 assay targets a highly conserved region of the pol gene.
- The Abbott RealTime™ HIV-1 assay offers the advantage of an automated instrument system for sample extraction, PCR MasterMix preparation, RNA dispensing and RealTime PCR amplification and detection:
 - increased efficiency & throughput
 - reduced "hands-on" time
 - broad dynamic range
 - robust performance on genetically divergent HIV-1 strains