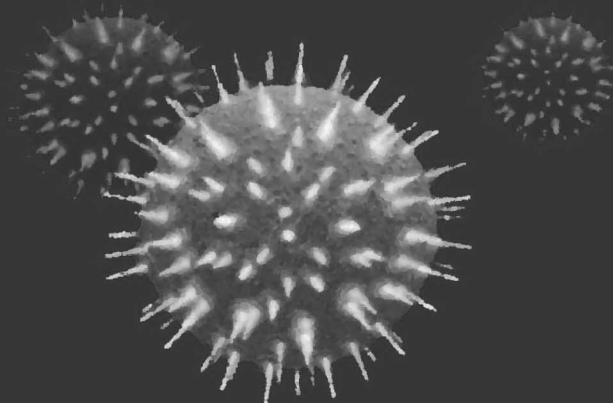


CRITICAL ISSUES IN THE TREATMENT
AND MANAGEMENT OF HIV INFECTION

REVIEW OF HIV-1 VIRAL LOAD TESTING



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REVIEW OF HIV-1 VIRAL LOAD TESTING

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INTRODUCTION

During the last several years, quantitation of plasma associated HIV-1 RNA or viral load (VL) has been validated in clinical practice and clinical trials as an important surrogate marker of HIV-1 disease progression, and reductions in viral load have been able to distinguish differences in potency between antiretroviral (ARV) regimens and the durability of treatment responses. In the evaluation of new ARV agents, plasma viral load levels are now a required test to assess the efficacy of new antiretroviral drugs and drug treatment regimens. Based on natural history studies and clinical trials, guidelines for the use of viral load testing in adult and pediatric clinical practice have been established (1, 2).

Several methods to quantify VL have been developed and are briefly reviewed below. Three methods are currently U.S. FDA approved for the assessment of HIV-1 disease progression risk or antiretroviral treatment response. Additional methods are approved to screen blood products for HIV-1 infection. Although U.S. FDA approval does not include an indication to use viral load testing in the diagnosis of HIV infection, U.S. HHS guidelines suggest the use of viral load testing in those patients with symptoms consistent with acute HIV infection, and in whom HIV antibody tests are negative or inconclusive (1).

This update will review the current testing methods and applications, recent controversies associated with viral load testing and new technologies for viral load measurement that are in development.

CURRENTLY AVAILABLE ASSAYS

The three main types of viral load assays currently approved for clinical practice are listed in Table 1 and reviewed elsewhere in detail (3):

(i) HIV RNA reverse transcription-polymerase chain reaction (RT-PCR) (Amplicor™ HIV-1 Monitor versions 1.0 and 1.5, Roche Diagnostics). Both versions (1.0 and 1.5) are available in a standard format, which has a linear range between 400-750,000 copies/mL and an ultrasensitive format, which has a linear range between 50-75,000 copies/mL. This assay involves target and co-amplification of an internal standard together with sample HIV-1 RNA using RT-PCR. Recent advancements include the use of an automated robotic system for amplification and detection/quantitation (COBAS AMPLICOR™), automated nucleic acid extractions machines (MagNA Pure LC™* and COBAS AmpliPrep™) and under development, a real-time PCR assay (COBAS TaqMan), which will extend the dynamic range of the current assay.

* The MagNA Pure LC™ is not FDA-approved for sample preparation for HIV.

(ii) Branched chain DNA (bDNA; Versant™ HIV RNA 3.0 Assay, Bayer Diagnostics) measures the amount of target nucleic acid bound to single-stranded probe by DNA following signal, not target amplification with bDNA. It has a linear range of between 75-500,000 copies/mL.

(iii) Nucleic acid sequence-based amplification (NASBA) or NucliSens™ HIV QT or NucliSens Easy Q® (bioMérieux) utilizes the co-amplification of standards and sample by means of reverse transcriptase, T7 polymerase, and RNase H-mediated reactions with primers conjugated to a T7 promoter. It has a linear range of between 40 – 10,000,000 copies/mL. The new Easy Q assay uses real-time NASBA amplification and molecular beacon detection technology and has a range of 50 to 3 million copies/mL (4).

(iv) Newer assays. The LcX® assay (Abbott) also quantitates HIV-1 RNA in plasma using competitive RT-PCR followed by a microparticle enzyme immunoassay (EIA), and includes an internal control. The dynamic range is between 50-1,000,000 copies/mL. The LcX assay compares favorably with currently available assays, but produces somewhat higher copy number (5, 6).

Another approach is to measure HIV-1 reverse transcriptase activity. The ExaVir® Load (Cavidi) is quantitative HIV-RT assay measures the amount of DNA made by HIV-RT from virions contained in plasma by a process similar to standard EIA tests. The DNA produced in femtograms/mL is then converted to copy number/mL (7). In recent studies, RT activity was highly correlated with viral load copy number from currently available assays (8). It has a linear range between 400 to 1, 250,000 copies/mL, and although a longer assay time is required, is somewhat less costly than traditional viral load tests.

PERFORMANCE ISSUES

Although these assays all measure the same input target, difference in assay standardization, sample preparation, assay methodology, dynamic range, and sensitivity threshold all contribute to variation in derived copy number.

Major assay types have been shown to be accurate, reproducible, and yield comparable copy number in clinical studies (3, 9, 10). Plasma HIV RNA assay variability is caused by both technical and biological factors. Technical variability, which is the standard deviation of testing the same sample multiple times, is about 0.2 log₁₀ copies/mL (3). Biological variability, which is the standard deviation of testing plasma samples obtained over brief periods of time from patients with stable treatment (or no treatment), is about 0.4 log₁₀ copies/mL (11). However, the results from different assays may differ by as much as 0.5 log₁₀/mL and therefore clinicians should not test samples from the same patient using different assays (9). Further, it is important to consider HIV-1 subtypes in the performance of these assays. In general, current versions will detect and quantitate almost all M type viruses, although subtype O and recombinant strains may be detected, but not be accurately quantified. The LcX assay appears to quantitate subtype O more accurately (6, 12).

For each of the commercially available assays, plasma should generally be collected in tubes containing the anticoagulant EDTA, either in standard blood collection tubes or plasma preparation tubes (PPT). Acid citrate dextrose (ACD), or in the case of the NucliSens assay,

heparin-containing tubes can also be used. Although previous data indicated a general equivalence in viral load results collected in different tube types, recent data suggests that viral load results derived from different tube types may not be equivalent.

In a recent clinical trial, critical differences in the number of subjects who achieved and sustained undetectable viral loads were seen when plasma was collected in standard EDTA compared to PPT tubes (13). Technical differences in centrifugation time and speed as suggested by the Amplicor assay and PPT handling instructions probably contributed to these differences. The significance of these findings for clinical practice is unclear, but may contribute to intermittently detectable viral loads (see blipping below).

Although current assays recommend that plasma should be separated and frozen at -20°C to -70°C within 4-6 hours of collection to insure maximum stability of RNA, recent studies indicate that valid and reproducible results can be achieved utilizing dried whole blood or plasma spots on filter paper, or larger volumes of plasma on a cellulose acetate matrix (14, 15). This would eliminate the need for immediate freezer storage and expensive shipment to referral laboratories and advance the use of these assays in resource poor areas.

DIAGNOSIS AND NATURAL HISTORY OF HIV INFECTION

Plasma HIV RNA levels are extremely high during primary HIV infection and prior to seroconversion, ranging from 10^5 to 10^7 copies/mL (16). After seroconversion, viral load usually drops 1-2 \log_{10} /mL as equilibrium develops between virus replication and immunologic containment. This steady-state level is highly variable among patients and is strongly predictive of disease progression (17, 18).

In general, after seroconversion, women have viral loads that are one third lower than men, and men who are infected through heterosexual sex or injection drug use have viral loads that are half of the viral load of men who have sex with men (MSM) (19). However, in both men and women, plasma HIV RNA increases slowly over time (about 0.1 \log_{10} /mL per year) and in patients with CD4 counts <100 cells/ mm^3 , the plasma HIV RNA levels of women are comparable to those of men.

Some have argued that a lower plasma HIV RNA threshold should be used for starting antiretroviral therapy in women, but this is not standard practice. Although there are significant differences in these viral loads, there is no difference in survival when gender, risk factor, or age is considered. HIV viral load levels are also correlated with the risk of sexual transmission. Recent studies found that risk of transmission was highly correlated with plasma viral load and that HIV infection was unlikely when their partner's plasma viral load was $< 1,500$ copies/mL (20, 21). Viral loads in women do not vary over the course of a menstrual cycle (22). In general, pregnant women with HIV infection do not have higher viral loads than non-pregnant women (23). However higher blood viral loads during pregnancy and in breast milk post-partum are associated with greater risk of transmission (24).

Viral load levels in the neonatal period can be significantly different depending on whether HIV infection occurred *in utero* or during the peripartum period. The viral load at birth and one month after birth is much higher in those babies infected *in utero* (25). By one month after birth, viral

load quantitation is very sensitive for diagnosis and the sensitivity is not affected by maternal zidovudine use, although the viral load level in those infants that are infected is lower (26). In general, infants have a rapid rise in viral load over the first 1-2 months, followed by a slow decline over the next 2 years. In contrast to adults, who experience a reduction in viral load concomitant with seroconversion and development of a cellular immune response, children usually maintain viral load levels that are $>10^5$ copies/mL during the first year of life (27).

In general pediatric viral loads tend to be higher than in adults, perhaps reflecting a more immature immune system and an inability to contain viral replication. The prognostic value of viral load in infants and children appears to parallel that seen in adults. A high viral load level at 4 weeks of age and/or maintained out to 6 months in the absence of therapy was highly predictive of disease progression within the first two years (2). Higher maternal viral load at delivery is also associated with neonatal viral loads and disease progression (28).

Finally, several studies have suggested that active infections such as tuberculosis, herpes viruses, or other opportunistic infections may cause acute increases in plasma HIV RNA levels of $>0.5 \log_{10}$ copies/mL, primarily in untreated patients or antiretroviral (ARV) treated patients with poorly controlled virus replication (29, 30). Hepatitis C virus (HCV) co-infection is also associated with 2-fold or greater HIV viral load levels and lower likelihood of achieving sustained undetectable viral loads (31). Immunization with standard vaccines such as those for influenza can also result in transient increases in viral load in some patients and has been reported rarely to result in the appearance of new ARV resistance mutations (32, 33). However, the benefit of vaccination far outweighs such small risks of transient increases in viral load.

VIRAL LOAD MONITORING DURING TREATMENT

Many studies have demonstrated rapid and sustained declines in viral load after starting HAART. In almost all treatment-naïve patients, HAART results in a 2-3 \log_{10} copies/mL reduction in viral load. A viral load of <50 copies/mL can usually be achieved within 8-16 weeks of initiating therapy. This may be contingent on the baseline viral load level, the potency and number agents used in the regimen, and the treatment experience of the patients. Although patients with viral loads $>100,000$ copies/mL appear to take longer in achieving undetectable viral loads, they are just as likely to do so (34). The nadir level of viral load has been found to correlate with the duration of treatment response (35). Those patients who do not achieve a viral load of <50 copies/mL are more likely to demonstrate virologic failure within the first year of starting treatment (36). In addition, achieving a viral load $<1,000$ copies/mL after 4 weeks of treatment was also found to be highly predictive of having sustained viral load suppression (37).

Recent observations have found that patients receiving HAART, and who have achieved undetectable viral loads (<50 copies/mL), are found to have low level detectable viral loads (usually <500 copies/mL) periodically. This phenomenon has been referred to as blipping. Recent longitudinal studies have indicated that patients manifesting blipping are not more likely to experience virologic failure than those patients with sustained viral load suppression (38). A recent intensive study of viral load monitoring in those patients with stable undetectable viral loads, indicated that most patients demonstrate blipping, and that it was

not associated with the detection of new ARV resistance mutations. Further, this study compared viral load testing performed in two different laboratories and found that the blips were not concordant between laboratories, indicating that these low level detectable viral loads are more likely the result of laboratory based phenomenon and not the new onset of viral replication or development of ARV resistant escape mutants from sanctuary sites (39).

Consistently detectable viral loads above the lower limit of detection are more likely to represent virologic failure than transient blips (40). Upon discontinuation of ARV treatment, viral load rebound is variable and is dependent on a number of factors including whether the viral load was undetectable or not at the time of interruption, presence of ARV resistant virus, CD4 count at pretreatment and at interruption, and the number of prior interruptions. Interruption in patients successfully suppressed, was recently shown to result in viral loads at least 0.5 log₁₀/mL below pretreatment values (41).

Reinitiation of ARV treatment in those patients previously suppressed and in whom ARV resistance is not present, usually results in successful resuppression (undetectable viral loads) (42). In those patients with evidence of ARV resistance, viral load response and chance for suppression is variable and depends on the length of interruption, current CD4 count, and number of ARV medications used upon reinitiation of treatment (42-44).

Criteria have also been established for viral load thresholds that define when treatment should be initiated or in the case of treatment failure, indications to change regimens. Current guidelines suggest that ARV treatment should be started in those patients with CD4 counts < 350/mm³ with any detectable viral load and in those patients with CD4 > 350/mm³ and viral loads > 100,000/mL (1).

Table 2 lists the indications for viral load testing. In general, those patients who have not achieved a 0.5 to 0.75 log₁₀ copies/mL reduction in viral load by 4 weeks, a 1 log₁₀ copies/mL reduction by 8 weeks, or an undetectable viral load (<50 copies/mL) by 15-24 weeks should be considered for a treatment change or modification. Patients who achieve an undetectable viral load (<50 copies/mL) but experience a rebound in viral load to detectable levels should first have the viral load confirmed. If the viral load rebound is within 50-5,000 copies/mL, current guidelines suggest that short-term observation, rather than an immediate change in treatment could be recommended. If viral load rebound is greater, or after initial observation increases to >5,000 copies/mL, a regimen change is indicated.

SUMMARY

HIV-1 viral load quantitation continues to be an important diagnostic and therapeutic test for use in clinical practice, clinical trials, and blood screening. New technologies will continue to improve accuracy, detection of more diverse viral subtypes, turnaround time and hopefully costs, so that areas with limited resources can use this tool as treatment becomes more available. Providers should remain aware of emerging trends (e.g. changes in assays, co-morbid conditions, treatment interruption, blipping, etc) that can affect viral load quantitation and therefore clinical decision making.

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Table 1. Summary of Viral Load Assays

Assay Name	Amplicor HIV-1 Monitor®	LcX® HIV RNA	Versant® HIV-1 RNA 3.0	NucliSens® HIV-1 QT	Retina™ Rainbow
Company	Roche	Abbott	Bayer	bioMerieux	Primagen
Assay Type	RT-PCR	RT-PCR	bDNA	NASBA	NASBA
Range (copies/mL)	S: 400-750,000* US: 50-75,000	50-1,000,000	75-500,000	50-1,000,000	500-50,000,000
Specimen Type	EDTA, ACD Plasma	EDTA Plasma	EDTA Plasma	EDTA, ACD, Heparin Plasma	EDTA, ACD, Heparin Plasma
Volume Required	S: 0.2 mL US: 0.5 mL	0.2 mL	1.0 mL	1.0 mL	0.2 mL
HIV Gene analyzed	gag	pol	pol	gag	LTR
Subtypes	Group M (All)	Group M (A-G), O	Group M (A-G)	Group M (All)	Group M (All)

*S = standard, US = ultrasensitive

Table 2. Indications for Plasma HIV RNA Testing*

Clinical indication	Information	Use
Syndrome consistent with acute HIV infection	Establish diagnosis when HIV antibody test is negative or indeterminate	Diagnosis
Initial evaluation of newly diagnosed HIV infection (together with CD4 counts)	Baseline viral load "set point"	Decision to start or defer therapy
Every 3-4 months in patients not on therapy (together with CD4 counts)	Changes in viral load	Decision to start or defer therapy
2-8 weeks after beginning therapy	Initial assessment of drug efficacy	Decision to continue or change therapy
3-4 months after start of therapy	Maximal effect of therapy	Decision to continue or change therapy
Every 3-4 months in patients on therapy*	Durability of antiretroviral effect	Decision to continue or change therapy
Within 2 weeks of a viral load blip	Detect early virologic failure	Decision to continue or change therapy
Clinical event or significant decline in CD4 count	Association with changing or stable viral load	Decision to continue, initiate, or change therapy

*Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents—April 7, 2005. <http://www.aidsinfo.nih.gov/guidelines/>