

HIV Viral Load Monitoring in Resource-Limited Regions: Optional or Necessary?

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(See the brief report by Bagchi et al. on pages 135–8 and the editorial commentary by Schooley on pages 139–40)

Although it is a standard practice in high-income countries, determination of the human immunodeficiency virus (HIV) load is not recommended in developing countries because of the costs and technical constraints. As more and more countries establish capacity to provide second-line therapy, and as costs and technological constraints associated with viral load testing decrease, the question of whether determination of the viral load is necessary deserves attention. Viral load testing could increase in importance as a guide for clinical decisions on when to switch to second-line treatment and on how to optimize the duration of the first-line treatment regimen. In addition, the viral load is a particularly useful tool for monitoring adherence to treatment, performing sentinel surveillance, and diagnosing HIV infection in children aged <18 months. Rather than considering viral load data to be an unaffordable luxury, efforts should be made to ensure that viral load testing becomes affordable, simple, and easy to use in resource-limited settings.

BACKGROUND

Scaling up antiretroviral therapy in resource-limited regions requires a simplified approach [1]. Because of inadequate laboratory capacity, many programs have minimized laboratory monitoring [2] in an effort to accelerate widespread availability of HIV treatment. In particular, the measurement of HIV-1 RNA levels (i.e., viral loads)—which is done routinely to monitor HIV-infected patients in high-income countries—was not recommended

for use in resource-limited settings in the 2003 World Health Organization (WHO) guidelines [3]. Equipment to determine the viral load is often unavailable or sits unused. Where available, viral load testing is prohibitively expensive: a single viral load test costs \$20–\$160 (in US dollars). Because the majority of HIV-infected people are still unable to access treatment, and because funding remains limited, it has been argued that resources should be applied to prevention measures and to the initiation of treatment, rather than to performance of expensive laboratory tests used to monitor patients who are already receiving treatment [4].

There are, however, a number of important reasons to implement viral load testing in resource-limited settings. Nearly 1.5 million people worldwide are receiving antiretroviral treatment, and this greatly

increases the need to detect cases in which first-line treatment has failed. More than 800,000 infants are newly infected with HIV each year, but infection cannot be readily diagnosed without viral load testing. As the need for viral load testing increases, technologies to determine the viral load are becoming simpler, and costs are decreasing [5]. Given the \$8.3 billion annual investment in HIV treatment in resource-limited settings [6], the question of whether high-quality, effective HIV care can be provided without viral load monitoring needs to be revisited. The focus of this article is the clinical use of viral load to manage antiretroviral treatment and to diagnose HIV infection in infants. Other uses, such as monitoring the level of drug resistance in a population and assessing the quality of a treatment program, are also outlined.

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USE OF VIRAL LOAD MEASUREMENTS IN HIGH-INCOME COUNTRIES

In high-income countries, determination of the CD4 cell count and viral load is used to determine whether antiretroviral treatment is indicated, and viral load data are used to gauge whether antiretroviral treatment is successful [7, 8]. When viral replication is suppressed to low levels, resistance mutations cannot emerge, and a durable treatment response ensues. Viral replication in the presence of antiretroviral treatment favors selection of resistance mutations and treatment failure. Viral load assays and drug resistance tests are used routinely in high-income settings to guide most treatment decisions.

Viral load measurements are optimally used to guide treatment when 2 conditions are met. First, effective plasma drug levels must be assured. It would be erroneous to conclude that treatment has failed if the patient's adherence is poor or if the regimen has not been taken as prescribed (adherence is not the only issue; drug quality, bioavailability, and drug interactions can be detrimental). Second, alternative drugs must be available. In the absence of alternatives to a failing regimen, the use of resources for viral load testing is ill advised. In high-income countries, >20 antiretrovirals are now available, providing options for second-, third-, and even fourth-line ("salvage") regimens, and new molecules, such as third-generation protease inhibitors, integrase inhibitors, and CCR5 inhibitors, will further expand the possibilities for salvage therapy. However, only a few second-line drugs are available in resource-limited settings.

Although there is a consensus on the triggers for initiation of treatment (CD4 cell count, 200–350 cells/ μ L) and on the viral load targets during treatment (the viral load should be "undetectable," meaning that the HIV RNA level is <50 to 400 copies/mL, depending on the assay's sensitivity), opinions diverge on the critical issue of the appropriate response to low-

level viremia in patients who are receiving treatment [7, 8]. In high-income settings, some clinicians interpret any sustained, detectable viremia (viral load, >50 copies/mL) as treatment failure that necessitates a switch in the regimen, because some resistance mutations are likely to emerge even with low-level replication. Others take a more flexible approach, weighing clinical and immunologic measures of treatment success, especially in heavily treatment-experienced patients whose options for active antiretrovirals are limited. Also, for any given viral load, the decrease in the CD4 cell count is slower in patients who are infected with drug-resistant HIV than in those who are infected with wild-type HIV [9]. In some triple-class-experienced patients, CD4 cell counts may remain stable for months or years, provided that the viral load does not exceed 10,000 copies/mL [10–12].

POTENTIAL BENEFIT OF VIRAL LOAD MEASUREMENTS IN RESOURCE-LIMITED SETTINGS

Treatment failure. Establishment of a viral load threshold to guide treatment switch decisions in resource-limited settings is fraught with challenges. In most national protocols, the options after a failed first-line regimen are generally limited to a single second-line regimen. The prices of second-line regimen drugs are currently 10-fold higher than the prices of first-line regimen drugs [13]. Viral load and resistance testing are rarely available, and where they are available, the cost and complexity of testing (which requires skilled technicians at a referral laboratory and sample transfer by cold chain) severely limit the ability to perform these tests routinely. Thus, the use of routine viral load monitoring in support of a developed-world strategy based on monitoring for maximal viral suppression has not been implemented in most resource-limited settings.

In the absence of routine viral load testing, treatment failure has generally been

defined by clinical criteria and CD4 cell count, and the revised 2006 WHO guidelines suggest a number of possible algorithms [3]. However, changes in CD4 cell counts are difficult to interpret as a result of individual variations in the immunological response to antiretroviral treatment. An algorithm based on clinical history, hemoglobin level, and CD4 cell count has recently been proposed, but it has not been validated in routine clinical care [14]. In fact, no validated definition of immunologic treatment failure based on the CD4 cell count exists, and there are no data on the long-term outcome of CD4 cell count-guided treatment changes. A recent study from Botswana suggests that the utility of CD4 cell count data to predict virological failure is limited [15]. Because clinical failure is an even later development, defining treatment failure on clinical grounds alone is equally suboptimal.

In the few settings where viral load testing is available, cost issues have modified the way it is used to approach treatment failure. A decreasing CD4 cell count triggers concern about treatment failure, and the viral load is determined to assess virological failure only in patients with decreasing CD4 cell counts. In principle, a predetermined threshold of virological failure would then trigger a switch in therapy; in practice, decisions are made on a case-by-case basis. Complicating this approach, studies in resource-limited settings have shown frequent discordance between virological and immunological responses to antiretroviral treatment, including a marked increase in CD4 cell counts in patients without complete viral suppression or a decrease in CD4 cell counts in patients with an undetectable viral load [16].

Thus, 2 critical questions remain. First, if viral load test results provide an early sign of treatment failure and predict clinical outcome, can a single viral load threshold be used to determine when to switch from a first-line to a second-line regimen in resource-limited settings? And,

if so, can a viral load test be made affordable and practical for this use?

The answer to the first question depends on the biology of virological failure and the availability of second-line drugs. The longer that viral replication occurs under ineffective plasma drug levels, the more likely it is that mutations will accumulate, jeopardizing future treatment options. If programs had 2 sequential regimens with limited cross-resistance available, resistance testing would not be needed for at least for some years after failure of the first regimen. Currently, failure of the WHO's recommended first-line regimens—2 nucleoside reverse-transcriptase inhibitors plus 1 nonnucleoside reverse-transcriptase inhibitor—is associated with thymidine analogue mutations, the M184V mutation, and nonnucleoside reverse-transcriptase inhibitor mutations. A second-line regimen of 2 new nucleoside reverse-transcriptase inhibitors (abacavir, didanosine, and/or tenofovir), combined with a boosted protease inhibitor, can be expected to retain reasonable activity. Nonetheless, data from a cross-sectional study from Malawi of 50 patients who had been exposed to stavudine, lamivudine, and nevirapine and who had viral loads >1000 copies/mL revealed a highly predictable mutation pattern without thymidine analogue mutations (although the median duration of follow-up in this cohort was only 8.3 months) [17]. Greater access to an expanded, low-price, second-line formulary that has minimal cross-resistance with the first line, combined with rational use of viral load testing, would be sufficient to minimize drug resistance and maximize the likelihood of success for patients whose WHO-recommended first-line regimens fail.

Studies can also address the second question: is there a single threshold or a narrow range of viral loads that can be used as a trigger for treatment switches? Evidence from the PLATO Collaboration and other studies suggests that, as long as the viral load remains <10,000 copies/mL, CD4 cell counts remain stable and the risk

of clinical progression is low [14–16, 18]. Some national programs have set 5000 copies/mL as a threshold for switching regimens. Additional virological data from ongoing clinical trials and observational cohorts in resource-limited settings, combined with genotype data from samples from patients with low-level viremia, will help to evaluate different thresholds and guide the rational use of viral load testing. Clearly, however, a qualitative test with a cutoff value of 10,000 copies/mL would be of immediate practical use.

The interpretation of low viral load data using this approach warrants comment. In high-income countries, the significance of viral loads of 50–1000 copies/mL continues to be debated, and the concept known a “blip”—transient, low-level viremia that returns spontaneously to an undetectable level without apparent clinical consequences—has been introduced [19, 20]. The interpretation of low-level viremia in resource-limited settings may be even more challenging because of the high rates of comorbidities, such as tuberculosis, malaria, and other common infections, that may induce blips [21, 22]. However, with an assay that has a cutoff value of 10,000 copies/mL, such blips would appropriately pass unnoticed. Nonetheless, the nature of blips in resource-limited settings is a research priority.

Adherence monitoring. In Khayelitsha, South Africa, antiretroviral treatment has been accessible since 2001; by April 2006, >3500 adults had started receiving therapy [23]. Viral load and CD4 cell count monitoring are routinely performed at baseline, at months 3 and 6 after the commencement of treatment, and every 6 months thereafter. Adherence support includes preparedness counseling, pillboxes, support groups, and mandatory disclosure to at least 1 “treatment buddy.” Viral load testing is also used to identify patients who need more-intensive adherence support. When a patient with a viral load >400 copies/mL is identified, he or she undergoes a cycle of adherence checks, pill counts, and weekly counseling sessions for

4 weeks, after which time the viral load is reassessed.

After 4 years of follow-up, 70% of patients continued to received their original regimens, with 17.9% migrating to a second-line regimen. In a sample of 598 patients, 515 (87%) were found to have an undetectable viral load at 3 months, and 416 (90%) were found to have an undetectable viral load at 6 months. With regard to the remaining patients with detectable viral loads, the ability of their viral load to return to an undetectable level correlated with the timing of detection of their virological escape and the subsequent intervention in adherence support provided. Only 25% of patients who were not reevaluated for ≥ 7 months were able to achieve an undetectable viral load, compared with 71% of patients who underwent an adherence intervention and a repeated viral load assessment within 4 months.

Thus, viral load testing combined with an adherence intervention may help patients with poor adherence to therapy maintain use of their first-line regimen, preventing unnecessary switches in treatment. Moreover, in settings with very high patient workload, viral load testing may allow staff to triage between patients who are eligible for self-administered antiretroviral treatment and patients who need more regular visits and support. The data from Khayelitsha are preliminary, and it should be noted again that broader implementation of strategies to use viral load data for early detection of non-adherence to treatment in resource-poor settings would require a simple, affordable viral load assay. Nonetheless, this approach is proving useful in a number of settings where viral load testing is available, including positive experience reported from programs in Nigeria (J. Wenkel, personal communication) Botswana (G. Brisson, personal communication), and Uganda [24]. Further evaluation is warranted.

Diagnosis of HIV infection in infants. In most resource-limited settings, children

Table 1. Specifications of existing viral load assays and target specifications for a viral load assay appropriate for resource-limited settings.

| Characteristic | Available viral load equipment/kit | Target requirements for viral load devices designed for resource-limited settings |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Assay characteristic | | |
| Sample collection method | Venipuncture | Fingerstick/heelstick ^a lancet |
| Sample volume, μL | 200–1000 | 100–200 ^a |
| Sample preparation | Three-step workflow, open system, ultracentrifugation, separate sample preparation area | Minimal (plasma and whole blood), closed system |
| Consumables per result | 1 blood collection tube, 1 needle, 1 micropipettor, 6 pipet tips, 1 pipettor, 2–4 pipets, reaction tubes, reagent tubes, reagent reservoirs | 1 lancet, 1 capillary collection tube, 1 disposable cartridge |
| Reagent characteristics | Refrigerated kits (2°C–8°C) | Reagents embedded on cartridge and stabilized to 40°C |
| Test cost | \$14–\$100 per result | <\$8 per result |
| Instrument characteristics | | |
| Power requirements | AC mains | Rechargeable battery |
| Characteristics | Multiple equipment components | Handheld/bench-top, single device |
| Instrument cost | \$30,000–\$60,000 | <\$1000 |
| Performance | | |
| Technician time | 10–60 min | <10 min |
| Time to result | 3–8 h | <2 h |
| Analytic/diagnostic range | Quantitative: from 50 to 10 ⁶ copies/mL; all clades | Semi-quantitative threshold: 10,000 ^a copies/mL; all clades |
| Training and skill level | Advanced training in molecular biology techniques | 1–2 days training, 10th grade education |

NOTE. AC, alternating current.

^a For diagnosis in infants.

born to HIV-infected mothers are tested with an antibody test to determine their HIV infection status. These tests are only conclusive after 15–18 months because of the potential for false-positive results associated with persisting maternal antibodies. Earlier identification of HIV infection in exposed infants and referral for anti-retroviral treatment are essential.

Detection of virus by nucleic acid amplification is the preferred method for diagnosis. Most experts agree that viral load testing performed when the child is aged 4–14 weeks is optimal; diagnosis of infection in breast-fed infants may require additional testing. Some programs have established routine, early infant HIV testing, in which at-risk infants are identified during regular postnatal follow-up visits (e.g., vaccination visits) and are tested as early as 4–6 weeks of age. DNA PCR is widely used, largely because it is cheaper than RNA PCR, although it is slightly less sen-

sitive [25–27]. Dried blood spots have proven to be useful as samples transported to a reference laboratory [28], but turnaround of results may still take several weeks, during which time many infants are lost to follow-up.

Trials from Malawi, Kenya, and Uganda have reported a 40%–50% mortality rate among HIV-infected infants within the first 24 months of life [29–31]. On-site, rapid diagnosis of HIV infection in infants would provide health care workers and caretakers with results during routine postnatal visits, allowing early treatment initiation where needed. It would also allow savings for overburdened health services by screening out as many as 90%–95% of exposed but uninfected children, limiting unnecessary use of trimethoprim-sulfamethoxazole, and guiding strategies for infant feeding in at-risk babies. For diagnosis of HIV infection in infants, as in monitoring in adults, a simple, quali-

tative viral load test with a detection threshold of 10,000 copies/mL would be more than sufficient [28].

Other uses: quality assessment, resistance surveillance, and vaccine efficacy studies. Viral load testing is also used in resource-limited settings to assess HIV treatment program quality, to monitor drug resistance, and to evaluate candidate vaccines. As a quality benchmark, the WHO has proposed that 70% of patients should achieve virological suppression (defined as a viral load <400 copies/mL) at 6 months of antiretroviral treatment in resource-limited settings [32–37]. A global network of drug resistance surveillance has also been established [32], and the US National Institutes of Health has begun planning for viral load testing in upcoming phase IIb and phase III vaccine trials (M. Schito and P. de Souza, personal communication). The availability of simple, affordable viral load tests

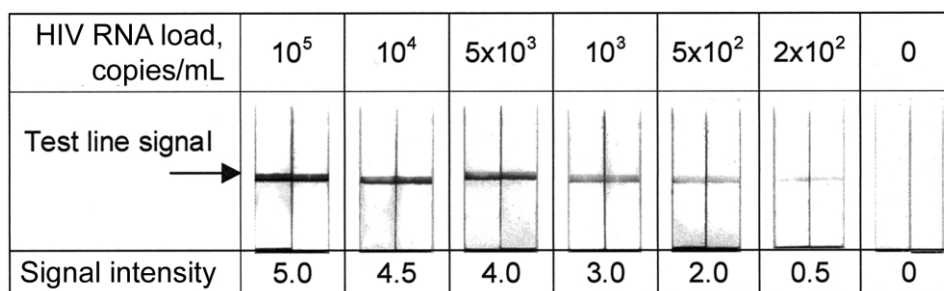


Figure 1. Prototype dipstick to quantify amplified nucleic acids for simplified viral load measurement in resource-limited settings (courtesy of Magda Dineva and Helen Lee, Diagnostics Development Unit, Cambridge University, United Kingdom).

would expand the reach of each of these programs [38, 39].

FUTURE ASSAYS FOR VIRAL LOAD MEASUREMENT IN RESOURCE-LIMITED SETTINGS

Four systems to measure viral load are now in use in high-income settings: the Abbott real-time HIV-1 PCR assay, the Bayer Versant HIV-1 RNA 3.0 (bDNA) assay, the bioMérieux NucliSens HIV-1 QT (NASBA) assay, and the Roche Amplicor HIV-1 Monitor 1.5 (RT-PCR) assay, which can also be run in a real-time format [40, 41]. Each system requires refrigerated reagents, multiple instruments, and isolation rooms to prevent cross-contamination. Highly skilled laboratory technicians proficient in molecular biology techniques and strong laboratory management are essential. Generic, low-cost reagents for real-time assays have been developed for use in resource-limited settings, with performance comparable to existing assays [42], and price reductions have been negotiated for commercial systems [5]. Although these developments are encouraging, because of the complexity of nucleic acid amplification assays, expansion of viral load testing capacity has been highly variable in low-income settings.

Two nonnucleic acid viral load detection methods have also been developed [43]. One, the ICD p24Ag assay, measures circulating HIV p24 protein after immune complex dissociation; this was originally developed as a research tool. The second,

the Cavidix ExaVir Load assay, uses a modified ELISA to measure reverse-transcriptase activity, which correlates with circulating HIV RNA levels. Although these are less complex than nucleic acid assays, both require refrigerated reagents, multiple instruments, and skilled laboratory technicians; moreover, the Cavidix RT ELISA test results are available only after 3 days [44]. Investigations of the use of p24 assays in clinical management in Africa have been disappointing [44, 45]. Clinical validation for both systems is incomplete.

Given the limitations of current tests for use in resource-limited settings, implementation of expanded viral load testing has been delayed, even in countries such as Brazil and South Africa, where laboratory infrastructure is more robust. Thus, in early 2006, Médecins Sans Frontières organized an expert consultation among academic experts and end users in resource-limited settings to identify the essential characteristics for a new viral load assay appropriate for resource-limited settings [46]. A survey was also performed among potential end users at 32 districts and local hospitals and clinics involved in the monitoring and care of ~18,000 patients in developing countries [47]. The critical specifications were established, including thresholds for adult monitoring and infant diagnosis, cost, automation, power requirements, and the technical demands on users (table 1).

Currently available assays do not yet meet these specifications. Several biotechnology developers are attempting to de-

sign new assays along these lines. As one example, scientists at Cambridge University have recently developed a new approach to quantify amplified nucleic acid using a simple dipstick [48] and a signal amplification system for visual detection at lower thresholds (figure 1). Other approaches to viral detection that can meet the specifications required of a viral load assay appropriate for resource-limited settings are also in development [49].

CONCLUSIONS

The revised WHO guidelines for HIV treatment in resource-limited settings released at the XVI World AIDS Conference in Toronto in August 2006 [3] specifically recognize the increasing use of viral load tests in many countries. For the first time, viral load data are considered in the criteria to define treatment failure, and viral load thresholds for resource-limited settings are suggested. These guidelines offer useful guidance for further research on this question. Although current assays remain expensive, viral load testing may prevent unnecessary switches to expensive second-line therapies, and the costs of not monitoring viral loads need to be considered.

The current practice in resource-limited settings of basing treatment decisions on CD4 cell counts and clinical signs can only lead to potentially dangerous delays and uncertain outcomes for a number of patients and widespread transmission of drug-resistant virus—in particular, in babies born to mothers with partial treat-

ment failure. With the increasing need for availability of second-line regimens, there is now a reasonable argument in support of the widespread, rational use of viral load testing. Although relatively expensive at present, it has the potential to prevent unnecessary switches to expensive second-line therapies, to assist adherence intervention programs, and to diagnose HIV infection in infants. We anticipate that advances in diagnostic technologies will lead to new viral load assays that will meet the specifications appropriate for resource-limited settings.

The focus in resource-limited settings has been almost exclusively on increasing access to drugs. Attention must now also be paid to monitoring to limit the costs associated with widespread use of expensive second-line therapy and to provide optimal treatment to patients.

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