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UNDETECTABLE

HOW VIRAL LOAD MONITORING CAN IMPROVE HIV TREATMENT IN DEVELOPING COUNTRIES

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EXECUTIVE SUMMARY

With more than eight million individuals worldwide now receiving antiretroviral therapy (ART), the ability to monitor and optimise treatment effectiveness is key to the success of HIV treatment programmes.

Viral load testing – measuring the number of copies of HIV in the blood – is the only way to accurately assess the level of viral replication in HIV-positive patients. Routine monitoring of viral load will help to reinforce a patient's adherence to ART alongside counselling and support, thereby ensuring viral suppression and preventing treatment failure before it occurs. Routine testing also ensures that health-care workers can diagnose treatment failure early on when drug resistance occurs, and appropriately switch patients from first-line ART to more effective second-line treatment regimens. With large numbers of patients in Africa already having been on treatment for several years, ensuring patients can access viral load testing is emerging as a global priority. Furthermore, for treatment as prevention to be successful, viral load monitoring will be a critical component.

For patients on ART, the World Health Organization (WHO) recommends viral load testing twice yearly in settings where testing is available. Unfortunately, viral load testing remains largely unavailable in resource-limited settings, in which the majority of HIV-positive patients reside. Instead of being an important routine monitoring and patient support tool in these contexts – as it is in resource-rich countries – viral load testing is rarely available, and where it is, its use is limited to confirming treatment failure. The result is avoidable morbidity and mortality among patients, and the potential transmission of drug-resistant forms of the virus.

It is critical, therefore, that access to viral load testing in resource-limited settings is prioritised as part of the next phase in the fight against HIV/AIDS. Poor access to viral load testing to date is a result of current test complexity, requiring specialised laboratory facilities. The majority of HIV-positive patients globally live in remote settings served by district-level laboratories that may be without reliable access to a power supply or highly trained staff, and where transport of samples to central reference laboratories causes delays. Poor access is also due to the fact that tests are costly. A lack of market competition to date means prices remain high. Ultimately, viral load testing prices will have to come down, as well as the cost of second- and third-line ART. Simple tests that can be performed at a community-based level using district laboratories, and/or a point-of-care test that can be performed at point of service, are now urgently needed.

This report by Médecins Sans Frontières (MSF) seeks to identify the next steps to improving access to viral load testing in resource-limited settings, by:

- Describing the importance of viral load monitoring
- Assessing the current state of play in terms of implementation of viral load testing in the developing world
- Exploring how to overcome technical barriers by looking at the research and development (R&D) pipeline and defining the ideal specifications of a viral load test for resource-limited settings
- Identifying strategies to overcome market barriers in order to make viral load monitoring more affordable.

With complexity and cost acting as the largest barriers to scale up, there is an urgent need to push forward the development and field validation of simple and affordable laboratory-based and point-of-care viral load tests.

To achieve this end, the following is proposed:

In the short- to medium-term: the HIV community must work to ensure that viral load testing becomes the basic standard of care. Donors should create incentives for more manufacturers to enter the market to increase competition and reduce prices. Strategies to reduce costs and to generate market competition should be explored. This includes: price transparency, pooling demand, analysing and removing patent barriers where they exist, and giving preference to 'open systems' to allow for greater competition on reagents and instruments. Operational research should be performed to accelerate the possibility of using a phased approach to replace immunological monitoring with virological monitoring, and diagnostic regulatory systems specific for resource-limited settings should be put in place.

In the medium- to long-term: Donors should fund the field validation and implementation of new tools for specific use in resource-limited settings and support their roll-out. Finally, future viral load test development should consider screening for key drug resistance mutations to support treatment switching decisions.

Funding the implementation of viral load should not be seen as a luxurious and avoidable expense, but should rather be recognised as a necessary and potentially cost-saving addition to current international commitments to scaling up treatment.

INTRODUCTION

Key to the success of HIV-treatment programmes is ensuring that ART is successful, and leads to constant and sustained suppression of HIV viral replication in patients such that their viral load is 'undetectable'.[i] Viral load monitoring, which provides that measurement, is the only way to obtain an accurate reflection of the magnitude of viral replication, and is a critical component in optimising treatment regimens.

Indeed, the World Health Organization (WHO) has recognised the importance of viral load testing since 2003, and their current guidelines for antiretroviral therapy in resource-limited settings recommend that viral load testing should be phased in wherever possible and performed every six months to ensure the appropriate and timely switch from first-line to second-line ART.[1] Furthermore, WHO and UNAIDS have established a working group, as part of the Treatment 2.0 initiative, tasked with guiding work on simplified and point-of-care diagnostics and monitoring tools for HIV, including: identifying which tests will be available; bottlenecks to the development and delivery of new devices; and developing implementation strategies.[2]

Although access to viral load testing has improved in recent years, it is still largely unavailable in resource-limited settings where the majority of the eight million patients taking ART reside.[3,4] Lack of access to viral load testing means that most treatment monitoring in resource-limited settings is done by clinical (observation of symptoms) or immunological (monitoring of CD4 counts) assessment. This assessment measures AIDS events and a decline in CD4-cell count, so is a measure of how the immune system has deteriorated. This is certainly not considered optimal, for both patients and public health.

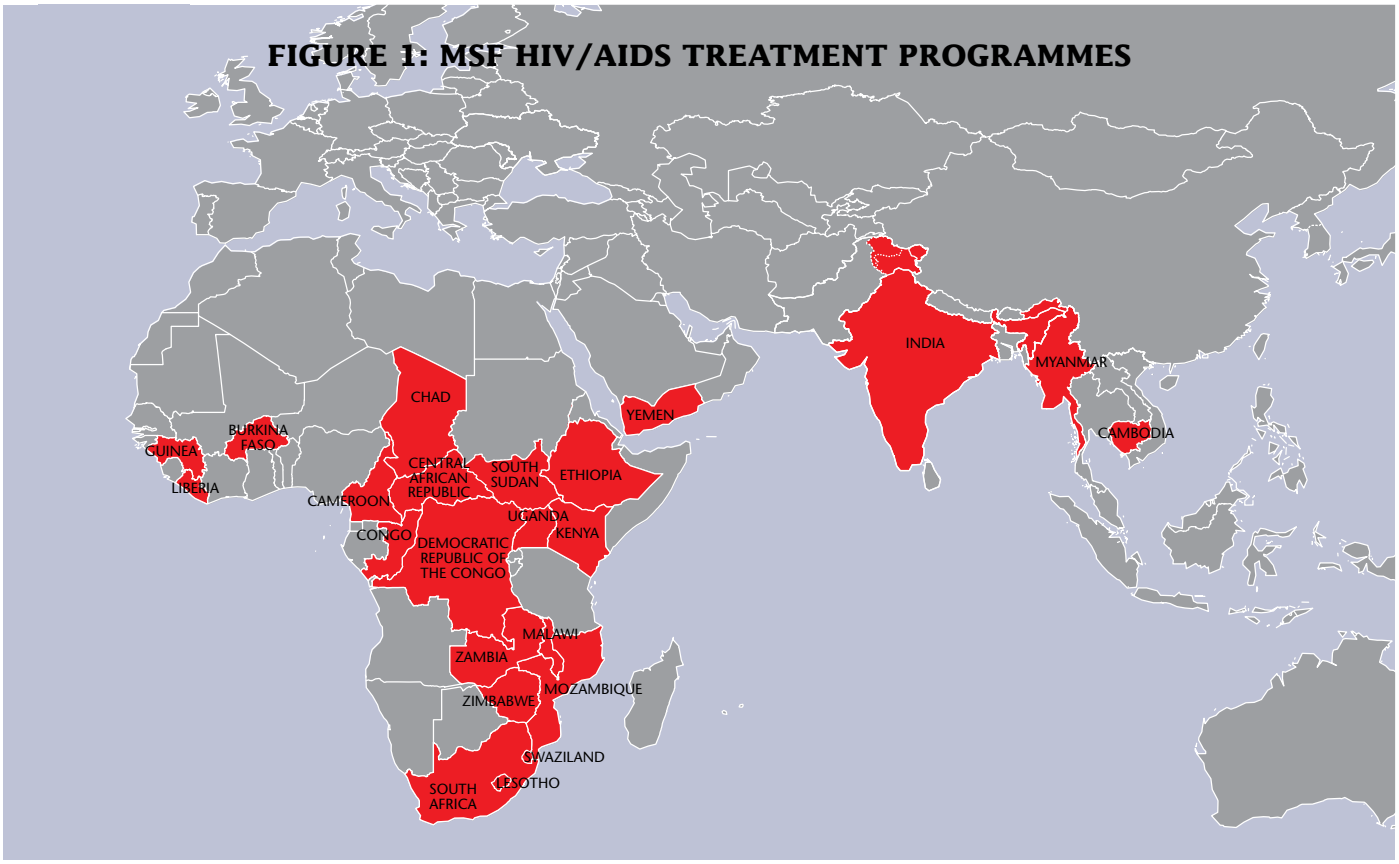
For patients, monitoring without viral load means increasing the risk of development of avoidable illness: patients using viral load testing have been shown to have better outcomes when compared to patients being tested by clinical and immunological monitoring, including more timely switching to second-line ART, and lower rates of loss to follow up and death.[5] Securing wider access to routine viral load monitoring is therefore essential. For public health, the presence of drug-resistant mutations following treatment failure after clinical and immunological monitoring is reported to be high.[6] This causes secondary resistance in these patients but also increased transmission of resistant viral strains when these patients infect other people. Overall primary resistance in sub-Saharan Africa is already at 5.6%, with Kampala, Uganda, and Yaounde, Cameroon, where treatment provision exceeds 10 years, at 12%.[7] This means that there is transmission of HIV-1 drug resistant virus to treatment-naïve individuals, especially in areas where there are long-term treatment cohorts. It is particularly worrying that drug-resistant mutations found in treatment-naïve individuals were found to be resistant to both first- and second-line drugs (NRTIs, NNRTIs and PIs).[8]

Fanelwa Gwashu is 40, and lives with her two children in Khayelitsha, where she runs a treatment adherence club. She has been on antiretroviral treatment for seven years and undergoes routine viral load testing to monitor her HIV treatment.

"To see if my treatment is effective, the clinic takes a blood sample to check my CD4 count and viral load. My latest viral load count, taken in June 2011, was undetectable. Antiretroviral treatment is life-long so it's encouraging to be told that the treatment is working well for me. It helps to know that whatever the difficulties, I am controlling the virus. I am proud that my viral load is undetectable, and I tell others about it. It helps me plan for tomorrow and I am confident I will live a normal life in the future."

The transmission of drug resistant virus is an escalating problem in longer-term treatment programmes run in resource-limited settings. This is in stark contrast to treatment programmes in high resource settings where, for example, in British Columbia, patients are infected with drug-susceptible strains and remain virologically suppressed on treatment.[9]

i. When a patient has fewer than 50 copies of HIV-1 RNA per millilitre of plasma (this value can be higher when a small sample volume is used).



ACCESS TO VIRAL LOAD IN MSF PROJECTS

As a medical organisation currently supporting ART provision to over 220,000 patients across 23 countries (see map), Médecins Sans Frontières (MSF) is confronted with the challenge of implementing viral load testing in the mostly remote places where we work.

In December 2011, an analysis of survey data on access to CD4 and viral load across 47 MSF projects in 15 countries was performed to investigate access issues in resource-limited settings. Selected countries included Cambodia, Cameroon, Central African Republic, Chad, Democratic Republic of Congo, Ethiopia, India, Kenya, Malawi, Mozambique, Myanmar, South Sudan, Swaziland, Uganda and Zimbabwe. Of these, 29 projects (61%) have some access to viral load. This is used mainly in a targeted way to confirm treatment failure following clinical or immunological failure before switching to second-line ART. In the majority of MSF's HIV programmes, there is no access to routine viral load monitoring. Some projects in South Sudan and Central African Republic still do not have access to any of these monitoring tools, not even CD4 counters.

Yet a recent internal review of MSF data from 12 countries found that only 2% of patients had ever received a viral load test result. This failure to adequately monitor patients on ART means that, for MSF programmes overall, fewer than 2% of patients have been switched to second-line ART when first-line ART is no longer working.[4] This is far lower than would be expected, and almost certainly means that a proportion of patients are failing treatment without being detected. Treatment failure, with the subsequent development of drug resistance, is going undetected in programmes where viral load testing is unavailable, and risks jeopardising the ability to treat patients in the future with existing treatment regimens.

In light of this poor access to viral load testing, MSF has recently implemented three different commercially available viral load testing platforms in three locations: the NucliSENS test (produced by BioMerieux) in Malawi, the Generic Viral Load Test (produced by Biocentric) in Swaziland and the ExaVir Load test (produced by Cavidi) in Myanmar, with plans to expand testing to other countries over the next few years.

With a view to the future introduction of newer and simpler tests, MSF is poised to roll out simple and affordable new pipeline viral load tests when they become available. MSF participated in the SAMBA (Simple AMplification Based Assay) viral load test (Cambridge University, Diagnostics Development Unit) clinical trials in Malawi and Uganda.[10] MSF is also piloting technologies, such as dried blood spots (DBS), which enable sample transportation over long distances and under harsh environmental conditions, including validating the use of fingerprick DBS to enable task-shifting to less-qualified staff.

From late 2012 onwards, implementation of viral load testing within MSF HIV programmes will be scaled up as part of a three-year project funded by UNITAID to improve the provision of routine viral load testing in decentralised, remote and resource-limited settings. MSF will compare the feasibility and cost-effectiveness of point-of-care testing versus district level laboratory testing for viral load through comparative operational research across eight sites in seven countries – Lesotho, Malawi, Mozambique, South Africa, Swaziland, Uganda and Zimbabwe. Best devices and best models of care will be selected from this implementation research. Furthermore, MSF will influence the price and accessibility of these tests through market assessment, price transparency, price reductions through negotiations with manufacturers and reduction of intellectual property (IP) barriers, working on target product profiles with manufacturers and advocating for the use of virological monitoring as the standard of care.

PART 1

WHY WE NEED ROUTINE VIRAL LOAD MONITORING

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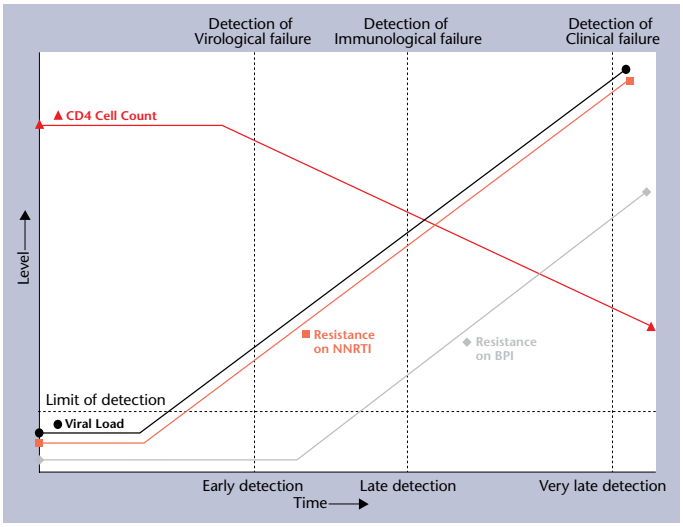
Routine virological monitoring has a significant role to play in ensuring that treatment is successful, for two main reasons.[11] Firstly, by detecting viremia early on, viral load monitoring helps reinforce targeted adherence counselling to prevent the development of drug resistance and treatment failure, and to re-suppress viremia if detected and managed early enough.

Secondly, once poor adherence has been ruled out, viral load monitoring serves to diagnose treatment failure early. It can guide a necessary switch to second-line ART and can be used to prevent prolonged viremia, patient morbidity/mortality and viral transmission.

Figure 2 illustrates how virological failure detects treatment failure and the development of drug resistance before immunological and clinical failure.

FIGURE 2: VIROLOGICAL MONITORING DETECTS TREATMENT FAILURE EARLIER



NNRTI: non-nucleoside reverse transciptase inhibitor; **BPI:** boosted protease inhibitor.
Source: Adapted from Bartlett J, Shao JF: Successes, challenges, and limitations of current antiretroviral therapy in low-income and middle-income countries. Lancet Infect Dis 2009, 9:637-649.

I. ADHERENCE COUNSELLING: VIRAL LOAD ENABLES EFFORTS TO BE TARGETED

Studies to date have shown that regular viral load monitoring is a necessary complement to patient adherence counselling to prevent treatment failure before it occurs.

Full and lifelong adherence to ART is essential to achieve constant and sustained viral suppression.[12–14] When ART concentrations are sub-optimal, for example because of poor adherence, viral replication selects for drug-resistant mutants because treatment only suppresses the replication of drug sensitive virus but not drug resistant virus. This allows drug-resistant mutants to replicate, which eventually leads to treatment failure.[15] A main benefit of routine viral load monitoring is therefore the ability to detect virological failure caused by poor adherence early on in these patients, and thus initiate intensive and targeted adherence counselling and support to prevent the development of resistant mutations (Figure 2).[16]

Routine viral load monitoring provides a simple and useful tool for monitoring treatment efficacy because it provides a cross-sectional indication of whether or not patients are stable or in need of adherence support. This targeted approach to adherence interventions can help direct a programme's resources to where they are most needed. This is especially important in prevention of mother-to-child transmission (PMTCT) programmes where such targeted adherence counselling to achieve virological suppression in pregnant women and lactating mothers on ART not only prevents drug resistance (and therefore treatment failure) but also PMTCT.

There are numerous practical challenges that may cause poor adherence – these include clinic accessibility, treatment affordability and pharmacy stock-outs. Furthermore, the high daily pill burden can lead to periods of imperfect adherence or selective drug taking.[17,18] Psychosocial factors, such as homelessness or drug-taking, can also decrease adherence to ART. A systematic review found that the most important barriers to adherence, reported across multiple settings, included: "fear of disclosure, concomitant substance abuse, forgetfulness, suspicions of treatment, regimens that are too complicated, number of pills required, decreased quality of life, work and family responsibilities, falling asleep and access to medicines".[19]

Support programmes are therefore a necessary part of adherence interventions.[20] Adherence counselling has been found to be an extremely efficient and cost-effective intervention, with fewer monthly health-care related costs and hospitalisations,[21,22] and should therefore be prioritised in all treatment programmes.

Despite many adherence obstacles, treatment adherence in sub-Saharan Africa has been demonstrated to be as good as, or better than, adherence reported in Western settings.[23,24] A recent systematic review indicated that the most successful adherence-boosting interventions in sub-Saharan Africa have included the use of treatment support tools such as mobile-phone text messages, diary cards and food rations.[25] These and other supportive (rather than supervisory) interventions are therefore a good way to improve adherence.[26]

While it is important to detect and correct adherence problems early on, long-term stable patients may not need to be monitored as frequently and a yearly viral load can be used as a tool to check for treatment efficacy. If the patients are found to be non-viremic (having no detectable viral load), no further follow-up is required. Non-viremic patients may also be eligible for self-administered ART, because no clinical intervention is required. This would greatly reduce the clinical workload.

VIEW FROM THE FIELD:
WHY WE NEED VIRAL LOAD TESTING FOR HIV

Dr. Steven Van Den Broucke, MSF HIV Coordinator in Zimbabwe from July 2010 to March 2012, explains how viral load testing has helped staff monitor HIV treatment. Viral load tests measure the amount of HIV in a person's blood; it is the gold standard in HIV treatment monitoring and regularly used in developed countries.

A viral load test indicates the number of copies of HIV's genetic material – ribonucleic acid (RNA) – per millilitre of blood. When there are less than 50[1] copies/millilitre in a person's blood, that person's viral load is said to be 'undetectable'. This does not mean there is no HIV in the sample, but that the virus has been suppressed to a level where the patient is able to stay healthy and is less likely to transmit the virus. Viral load tests prevent patients being unnecessarily switched to more expensive drugs or left to continue on ineffective therapy that can lead to drug resistance and ultimately death.

"MSF's HIV programme in Zimbabwe started in 2004. In April 2011, we started switching patients to a new tenofovir-based regimen and began to run viral load tests before the switch, in order to monitor patient progress. A year later, viral load monitoring became routine.

From a medical perspective, viral load is a critical tool to monitor how people are doing on treatment. If we run the test and find a patient with a detectable viral load, we know something is wrong. It can mean one of two things: either the patient is not taking their drugs or there is drug resistance, which means the medicines are not working.

We then work with a treatment counselor on what we call 'enhanced adherence', where we try to find out the underlying reason why the patient is not taking their medicine and see what we can do to support them. We try to stimulate and motivate patients to be adherent to their treatment and then we repeat the viral load test to see if we have managed to control the amount of virus in the blood.

After enhanced adherence, we see viral load significantly decrease in up to two thirds of patients. This means we were able to intervene at the right time with patients who were not taking their drugs regularly, before they developed a problem of drug resistance. Earlier detection of failure definitely gives better prognosis in the long run – it means the chances of having a normal life without disease is much higher.

In this way viral load is vital because before we had access to the test it was impossible to identify patients with poor adherence early and so we only became aware of the problem once they had already developed drug resistance. At this point it is too late to continue with the more affordable and easy-to-take first-line drug combinations.

So the big advantage of using viral load is not only to detect patients who are eligible for second-line treatment but also to keep patients on first-line treatment for as long as possible.

Dr. Steven Van Den Broucke, MSF HIV Coordinator, Zimbabwe

From a patient's perspective, it's a great thing to know your viral load is what we call 'undetectable'. It tells us that treatment is working and the chances of remaining healthy and keeping the virus under control in the long run are high, so it can really boost their self esteem and motivate patients to keep taking their medicine.

When a person is tested several months after they start treatment and their viral load is undetectable, it's one of the biggest motivators for a patient to keep taking their drugs.

Viral load also builds trust. If you can say to a patient that their viral load is undetectable, it creates a happy patient and a happy doctor and that enforces the relationship and trust between the two of them.

From the community perspective, there are also clear advantages of using viral load. If we manage to suppress viral load in the community, we will also manage to reduce transmission of the virus to other people. It has been shown that patients who are taking their drugs correctly and have an undetectable viral load are not infectious and therefore cannot transmit the virus to their partners. It means treatment can act as a prevention strategy, to reduce transmission to new people.

If we manage to get all the patients on ARVs to stick to their treatment, in the end, we will manage to win the battle against HIV and curb the epidemic by avoiding new infections."

1. This value could be higher when a small sample volume is used, such as a dried blood spot.

VIRAL LOAD AND TRANSMISSION OF HIV

An additional argument supporting the use of routine viral load monitoring is to ensure that viremic patients on suboptimal therapy are identified early, as this has been shown to prevent HIV transmission in the wider community.[27]

The importance of good adherence to ART is to completely suppress any viral replication so that viral load will be undetectable. If there is no virus in the blood then risk of transmission is greatly limited.[28, 29] Transmission is rare among HIV-infected persons with a plasma viral load below 1500 copies/ml.[30]

Following a landmark study assessing the potential prevention benefits of ART in 2011,[29] widespread, early provision of ART has been widely regarded as having high potential to make a substantial contribution to reducing HIV transmission. A recent model based on the IeDEA (International Epidemiologic Databases to Evaluate AIDS) Southern Africa database has shown that routine virological monitoring can lead to a 31% reduction in community viral transmission, and a subsequent reduction in HIV incidence.[31]

Experts have concluded that, for treatment as prevention to be successful, viral load monitoring will be a critical component.[32] Indeed, using viral load monitoring routinely in all HIV programmes ensures that HIV ART programmes are treating patients successfully at both the level of the individual, as well as the community.

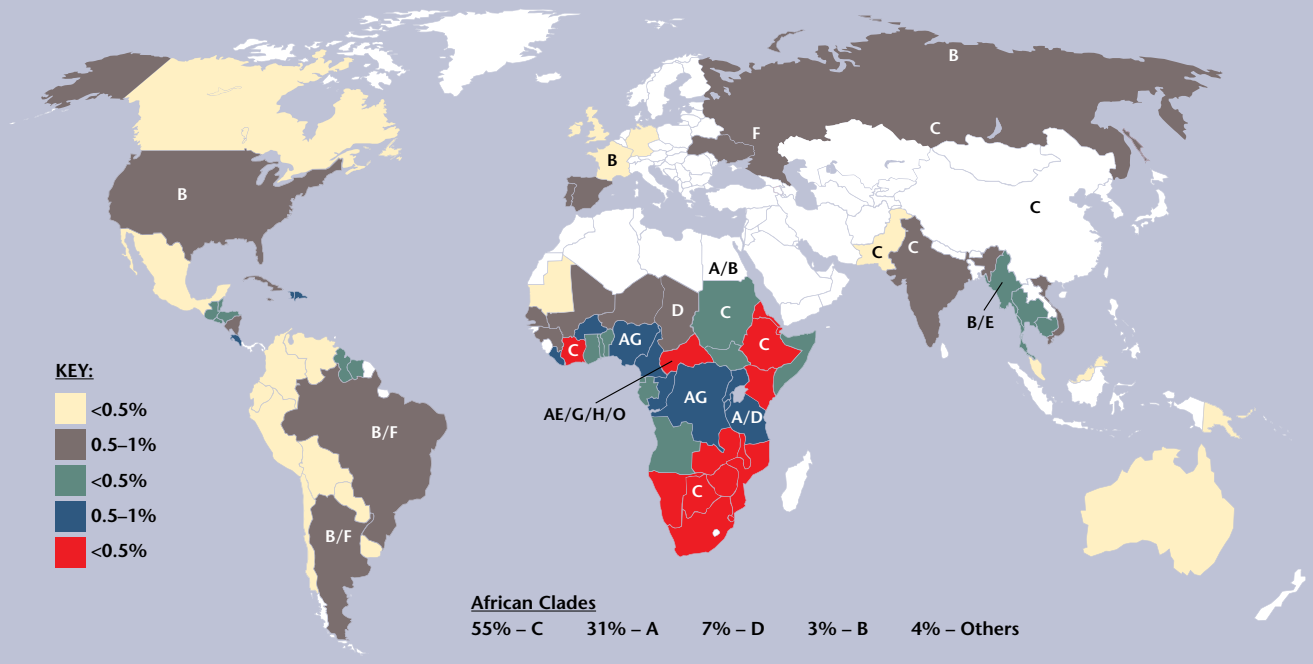
II. DRUG RESISTANCE: VIRAL LOAD HELPS TO CONFIRM TREATMENT FAILURE

Numerous studies show that regular viral load testing of patients enables treatment failure to be diagnosed early on when drug resistance occurs, so that patients can be switched rapidly to an effective alternative therapy. Observational studies of HIV-positive adults and children, for example, have found a significant benefit to using viral load monitoring over and above clinical and immunological monitoring alone,[5, 33, 34] improving the number of patients timeously switched to second-line ART because of failing first-line ART, and thus reducing mortality.

Virological failure is critical for the confirmation of treatment failure and, for first-line treatment failure, viral load results can effectively guide treatment change decisions. However, an additional test to measure drug resistance may be useful and cost-effective in cases where second-line failure is suspected.[35–39, ii] The value, in this case, is to adapt salvage regimens according to resistance patterns. MSF, for instance, uses genotyping to confirm the type of drug resistance following virological failure when switching from second- to third-line ART (when third-line is available), because it is the last treatment option and preserved use of second-line is therefore of paramount importance. An advantage of the genotyping assay designed by the CDC (Centers for Disease Control and Prevention) is that it may be performed on dried blood spots, which makes for convenient sample transport.[40]

ii. Direct detection of drug resistance may be measured through genotyping, which uses molecular assays to measure in vitro mutations, or through phenotyping, which measures the change in susceptibility of a clinical strain in an in vitro culture-based system.[117] Drug resistance testing is highly complex, requiring a specialised laboratory and skilled interpretation. This severely limits its use in resource-limited settings.

A GEOGRAPHICAL MAP OF HIV SUBTYPE PREVALENCE



Source: Source: Spira, S. 2003.[82]

LOOKING TO THE FUTURE: A VIRAL LOAD TEST ABLE TO DETECT RESISTANCE?

In models that chart the response to ART, viremia has emerged as the predominant predictor of drug resistance. For clinicians aiming to optimise treatment for patients in resource-limited settings, availability of drug resistance results may not, therefore, add a significant benefit over and above routine viral load monitoring alone.[41]

However, drug resistance testing could allow for the conservation of first-line treatment options if not all drugs in the original regimen need to be switched. A full switch to second-line ARVs may therefore be delayed or prevented through the use of targeted drug resistance testing.

A test that could simultaneously measure the presence or absence of key mutations would be very useful to identify patients requiring a switch to second-line therapy and informing subsequent drug choice for "targeted switching".

For example, mutations may be limited to one type of drug class in the regimen, and a targeted switch would then allow for the preservation of one or more drugs in the first-line regimen. Although many mutations exist, only a few exist at a high population frequency in a given setting, and it may therefore be easier, cheaper and simpler to design genotyping assays that measure only these so-called 'herald' mutations rather than a full complement of all possible drug mutations. Herald mutations have a greater than 10% global or regional prevalence, meaning that each mutation is present in greater than 10% of patients on ART.

Based on the evidence,[42–48] the following six mutations would be the most useful, clinically, to measure as key mutations:

1. **K103N** (resistance to efavirenz and nevirapine)
2. **Y181C** (resistance to efavirenz, nevirapine and, to a lesser extent, etravirine)
3. **G190A** (resistance to efavirenz, nevirapine and, to a lesser extent, etravirine)
4. **V106M** (resistance to efavirenz, nevirapine and, to a lesser extent, etravirine)
5. **M184V(/I)** (resistance to abacavir, emtricitabine and lamivudine)
6. **K65R** (resistance to abacavir, didanosine, emtricitabine, lamivudine, stavudine, and tenofovir)

In the absence of the availability of drug resistance testing, regional variant-type and drug resistance surveys should continue to be performed in order to monitor the type and prevalence of emerging strains and recombinants and drug resistance mutations.[49] Population level resistance testing is already being supported by the Global Fund and only a fraction of countries are taking advantage of this.[50] As new drugs are introduced, the core list of common mutations will need to be re-evaluated.

THE EVIDENCE BASE: THE ADVANTAGES OF ROUTINE VIRAL LOAD OVER CLINICAL OR IMMUNOLOGICAL MONITORING

A number of programme reports, both among adults and children, have found a significant benefit to using viral load monitoring over alternative monitoring methods – whether clinical or immunological.

Better treatment outcomes

A recent study compared outcomes in South Africa (18,706 adults), where routine viral load monitoring is performed, to Malawi and Zambia (80,937 adults), where only clinical and immunological monitoring is performed.[5] Over a three-year period following ART initiation, and even though the South Africa cohort had lower CD4 counts at the start of treatment (93 vs 132 cells/µl), all outcomes were significantly improved compared to those of patients in Malawi and Zambia, including CD4 count (425 vs 383 cells/µl), remaining on a failing first-line regimen (1.3% vs 3.7%), switching to second-line ART (9.8% vs 2.1%), loss to follow-up (9.2% vs 15.3%) and death (4.3% vs 6.3%).

The study concluded that this difference was likely due to the fact that viral load monitoring resulted in earlier detection of adherence problems, leading to targeted adherence counselling, and of treatment failure, leading to timelier switching to second-line ART.

Early adherence interventions have been found to be influential in long-term treatment outcomes. For example, in the MSF-supported programme in Khayelitsha, South Africa, a viral load test, combined with an adherence intervention for viremic patients, performed three months after ART initiation rather than six months, improves patient outcomes by achieving long-term virological suppression and avoiding premature treatment switches (total median follow-up between 1.6 and 3.6 person-years).[51] In the same programme, early adherence to ART also predicts long-term virological suppression and is the primary determinant of subsequent virological failure (five year follow-up).[52]

Continued above right

These studies underscore the importance of early viral load testing as a first step to prevent the development of drug resistance. Finally, increased disease progression is associated with viral load testing performed less than once per year.[53]

Better adherence support to prevent treatment failure

In Khayelitsha, South Africa, viral load monitoring along with adherence interventions has been successfully used by MSF to help patients maintain the use of their first-line ART regimen.[54] Once the viral load exceeds 400 copies/ml, a targeted adherence intervention is initiated (including four weeks of adherence checks, pill counts, and weekly counselling sessions), after which the viral load is reassessed. This intervention was successful in getting patients to take their medication and resulted in suppressing viremia in the majority of cases. Success depended on the early detection of viremia followed promptly by an intervention. A number of studies have thus highlighted the fact that viral load monitoring is a necessary complement to adherence counselling to trigger intensive and targeted adherence support to prevent treatment failure.

Similarly, in an MSF-supported programme in Kuchinarai, Thailand, 47 out of 51 viremic patients had undetectable viral loads following targeted adherence support[55] and, in two resource-limited communities in Cape Town (Gugulethu and surrounding areas), South Africa, viral load monitoring helped target adherence support to viremic patients, and to confirm treatment failure (to NRTIs and NNRTIs) in those patients where the adherence intervention failed.[45]

More timely and appropriate switching to second-line

An analysis of patient outcomes across 13 clinical sites in six different African countries showed that almost half the patients monitored by clinical and immunological means alone were unnecessarily switched to a second-line regimen while prolonged and undetected treatment failure was associated with extensive nucleoside reverse transcriptase inhibitors (NRTI) cross-resistance, highlighting the importance of viral load testing in the management of timely and appropriate treatment switches.[56] Viral load also enables more switches to second-line ART at higher CD4 counts,[57] diagnosing failures much earlier than CD4 monitoring, which has been shown to have a low sensitivity of predicting treatment failure. CD4 testing is only useful as a rule out test for treatment failure (with low positive but high negative predictive value).[58–61]

A study in India comparing the role of monitoring first-line ART using viral load as opposed to clinical/immunological methods alone showed that almost 25% of patients who met the WHO clinical/immunological failure criteria were, in fact, virologically suppressed.[34] This means that a quarter of patients would have been incorrectly switched to second-line treatment if not for viral load testing. This study highlights the important cost saving that can be made by having access to viral load, considering the cost implications of switching unnecessarily to more expensive second-line drugs.

Reduced development of drug resistance

A systematic review and meta-analysis of 10 studies from 11 countries (six from Africa) found that the frequent use of viral load testing for monitoring patients with a CD4 count <200 cells/µl was advantageous in reducing resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), NRTIs and thymidine analogue-associated mutations (TAMs). Resistance to all three was significantly less 48 weeks post-ART initiation in frequently-monitored versus infrequently-monitored patients.[33] This clearly demonstrates the importance of consistent viral load monitoring in the abrogation of drug resistance.

Better prediction of AIDS-defining events

Risk of HIV-related illnesses could be predicted, independently of clinical and immunological results, when children on ART were failing virologically (above 5000 copies/ml).[62] In pregnant Kenyan women, viral load was not a better predictor of mortality over-and-above CD4 monitoring alone,[63] but, in a different study, AIDS events could be predicted by viremia in treated patients with a CD4 count >350 cells/µl.[64]

Better monitoring of programme success

Viral load testing can also be used as an epidemiological tool for surveying the levels of viremia within an ART programme, and therefore monitoring the success of district and national HIV programmes at a population level.[65] For example, MSF measured both the detectable levels of viremia and the range and extent of drug resistance mutations present in a cohort of patients from Maputo, Mozambique, who had been taking first line ART for 12 months, to monitor programme quality and treatment efficacy.

PART 2

IMPLEMENTATION OF VIRAL LOAD IN DEVELOPING COUNTRIES

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I. LIMITED UPTAKE

Despite the advantages of routine viral load monitoring both as a trigger for targeted adherence support and to help establish drug resistance, the technology is not available outside of central reference laboratories. However, in the coming years, the majority of patients are likely to be managed in decentralized levels of care. Access to viral load testing is far less widespread than CD4 testing, mainly because the tests are more complex and expensive, requiring a specialised laboratory.[3,66]

When viral load testing is available, its use is usually restricted to confirmation of treatment failure. As a result, most treatment cohorts are monitored by clinical or clinico-immunological testing alone.

At present, only 76% of the WHO Africa-region member states have PCR-based facilities. While 84% of these countries can perform viral load testing, this is mainly confined to central facilities (50%), with regional (29%) and district (8%) capacity available to a lesser extent.

Of the six countries with the highest prevalence of HIV – Lesotho, Malawi, Mozambique, South Africa, Swaziland and Zimbabwe – the majority have not yet implemented routine viral load testing. Some only have limited targeted viral load testing available to confirm clinical and immunological failure – see Table 1. South Africa is the only country supplying routine viral load monitoring for all patients on ART, although Malawi has plans to add routine viral load testing gradually to reach universal access by 2015.

By contrast, all six countries have CD4 testing available, and start patients on ART at a CD4 count ≤350 cell/µl. All countries, except for Malawi, offer CD4 monitoring every six months for all patients on ART (Malawi does not monitor patients on ART using CD4). Although laboratory testing for CD4 is available in these high-burden southern African countries, only South Africa provides access to viral load testing.

TABLE 1: GUIDELINES IN COUNTRIES WITH THE HIGHEST HIV PREVALENCE

		GUIDELINES						
	HIV Prevalence	Latest country guidelines	CD4 for ART initiation		CD4 for ART monitoring		Viral load for ART monitoring	
			Universal eligibility	Frequency	Immunological failure	Frequency	Virological failure	Frequency
Lesotho	23.6%	2010	≤350 cell/µl	350-500: 3-monthly; >500: 6-monthly	WHO Guidelines	6-monthly	>5,000 copies/ml	Targeted for confirmation of treatment failure following clinical/immunological failure if available (only available for those funded privately or living in an NGO-supported area or living near the capital).
Malawi	11%	2011	≤350 cell/µl	6-monthly	WHO Guidelines	No CD4 follow up on ART	>5,000 copies/ml	6-months post-ART initiation and every 2 years thereafter; targeted for confirmation of treatment failure following clinical/ immunological failure if routine is not available (routine is being scaled up; currently only available for those funded privately or living in an NGO-supported area).
Mozambique	11.5%	2010 (with 2012 programmatic update)	≤350 cell/µl	6-monthly	WHO Guidelines	6-monthly	>10,000 copies/ml	6-months post-ART initiation; targeted for confirmation of treatment failure following clinical/immunological failure.
South Africa	17.8%	2010 (with 2011 programmatic update)	≤350 cell/µl	6-monthly	WHO Guidelines	6-monthly	>1,000 copies/ml	6-months and 12-months post-ART initiation and then annually thereafter.
Swaziland	25.9%	2010	≤350 cell/µl	6-monthly	WHO Guidelines	350-500: 3-monthly; >500: 6-monthly	>5,000 copies/ml	Targeted for confirmation of treatment failure following clinical/immunological failure.
Zimbabwe	14.3%	2010	≤350 cell/µl	350-500: 3-monthly; >500: 6-monthly	WHO Guidelines	6-monthly (when available)	>5,000 copies/ml	Targeted for confirmation of treatment failure following clinical/immunological failure if available (only available for those funded privately or living in an NGO-supported area).

In a recent analysis that pooled data from 46 MSF HIV programmes across 12 countries – Cameroon, Central African Republic, Democratic Republic of Congo, Ethiopia, Guinea, India, Kenya, Malawi, Mozambique, Myanmar, Uganda and Zimbabwe – only 2% of the 146,498 patients had at least one viral load result.

This is far less than what is needed, as shown by the fact that in programmes run in countries where routine viral load testing is state provided, such as at the MSF site in Khayelitsha (Cape Town, South Africa), 12% of patients who began ART in the last five years have been switched to second-line therapy. This inability to adequately monitor patients on ART in areas where virological monitoring is not available means that fewer than 2% of patients enrolled in MSF HIV programmes globally have been switched to second-line therapy.[4]

WHO/UNAIDS data for 2011 report that only 3% of patients on ART in low to middle-income countries are on second-line treatment.[67]

As such, virological failure, and the consequent development of drug resistance, is going undetected in programmes where viral load testing is unavailable, with affected patients suffering morbidity and death as a consequence, decreasing effectiveness of second-line therapy due to late switching, and potentially transmitting drug-resistant virus to other people in the community.

II. RECOMMENDATIONS FOR USE OF VIRAL LOAD MONITORING

WHO GUIDELINES

Based on a Cochrane review,[68] the current WHO guidelines[1] could be interpreted as not advocating enough for the uptake of routine viral load monitoring.

Current WHO guidelines:

1. Where available, use viral load to confirm treatment failure (strong recommendation, low quality of evidence).
2. Where routinely available, use viral load every six months to detect viral replication (conditional recommendation, low quality of evidence).
3. A persistent viral load of >5,000 copies/ml confirms treatment failure (conditional recommendation, low quality of evidence).
4. When viral load is not available, use immunological criteria to confirm clinical failure (strong recommendation, moderate quality of evidence).

The recommendations are rightly formulated to ensure that a lack of viral load monitoring does not form a barrier to starting ART. However, they have been misinterpreted by some funding agencies as implying that viral load monitoring should be delayed.[69] On the contrary, WHO recognised the importance of viral load monitoring as early as 2003, when it issued guidance expressing hope "that increasingly affordable methods of determining viral load will become available so that this adjunct to treatment monitoring can be more widely employed".[70]

FREQUENCY

Although it is evident that frequent and routine monitoring is necessary to prevent drug resistance, and therefore annual or biannual testing is suggested, further operational research is necessary to better define the ideal frequency for virological treatment monitoring in early, and stable, long-term patients. Research has gone some way in showing that the first monitoring result should be at three months post-ART initiation because it shows better virological and treatment outcomes than waiting six months post-ART initiation.[51]

VIROLOGICAL THRESHOLDS

Viremia acts as a trigger for intensive targeted adherence counselling. Once a patient has been found to be viremic, they should be enrolled in an intensive adherence support programme for three months, where after a second viral load test is done. If the results show virological failure, the patient will need to be switched. But the threshold that defines virological failure has yet to be properly established.

The current WHO-recommended threshold for virological failure has been set as two consecutive viral load tests measuring above 5000 copies/ml, with the first test having been followed by an adherence intervention. At the same time the guidelines state that the optimal threshold for defining virological failure has not yet been determined. This threshold was set because values above 5000 copies/ml are associated with clinical progression and a decline in CD4 cell count.[1]

MSF organised a meeting of experts to define the threshold and other specifications of viral load tests for developing countries.[54] It was suggested that a lower threshold of 1000 RNA copies/ml be used, which has more recently been recommended by others.[71]

Establishing a clear threshold is important as it defines how sensitive viral load tests have to be. Most quantitative viral load assays used in reference laboratories have a lower limit of detection of ~50 copies/ml, and an undetectable viral load is therefore defined as <50 copies/ml. Although optimal use of ART should render the virus undetectable, a threshold of 1000 copies/ml was proposed as a compromise between measuring virological failure too late to prevent the emergence of drug resistance and too early to prevent false alarm from isolated blips (transient increases in viral load), which are quite common in successfully treated patients.[72–74] Blips are clinically unimportant, and do not lead to drug resistance or treatment failure.[75,76] A threshold of 1000 copies/ml should then be high enough to avoid 'false alarms' but low enough to ensure that virological replication and drug resistance is detected early.

This information is important for manufacturers. A number of simple and inexpensive viral load tests that are in the pipeline are designed to be as low cost as possible, and to be used with a small volume of blood (e.g. fingerprick), but this has come at the price of sensitivity. Most platforms will not be able to detect low level viremia (50–1000 copies/ml) and there is some concern that, clinically, these tests may be insufficiently sensitive. Additional research is therefore required to define more accurately the clinically relevant cut-offs for virological failure, so that manufacturers have a clear objective when it comes to test design and performance.

CAN VIRAL LOAD IMPROVE EARLY INFANT DIAGNOSIS?

Measuring HIV DNA is the only way to definitely confirm an HIV infection in infants because the test measures the actual presence of HIV within cells (as pro-viral DNA incorporated into genomic DNA). HIV RNA testing may also be used for diagnosis provided that the infant has not recently been exposed to treatment. Rapid immunoassay tests, on the contrary, can only be used after 18 months of age, once maternal antibodies have been cleared (and provided that the mother is not breast-feeding and that the infant is not on ART). A definitive diagnosis of HIV infection in infants must be confirmed by two positive HIV DNA PCRs.[77]

Existing methods are cumbersome and involve considerable delays to diagnosis. Although the use of DBS has been used successfully to transport paediatric blood samples to centralised facilities far away from primary health care clinics, most HIV-exposed infants lack access to quick and reliable diagnosis.[78] Simpler point-of-care tests for EID are therefore urgently needed.

Although a diagnosis through the detection of HIV DNA is still the gold standard, in the event that an HIV DNA test is not available, or takes too long, or if there are doubts about the quality of the laboratory, a non-molecular diagnostic test may be considered to enable or expedite infant diagnosis.

One example of how paediatric diagnosis can be greatly simplified is through the measurement of p24 antigen as a proxy for viral RNA (this is not to monitor infants on treatment but rather to diagnose them for infection). The p24 antigen makes up the core protein of the virus and is present in plasma from the acute phase of infection onwards. Currently, only one assay for ultrasensitive p24 detection exists. Manufactured by PerkinElmer, the test is in the form of a one-day ELISA (enzyme linked immunosorbent assay) and therefore requires a laboratory and some technical skill to perform.[79] ELISA tests are feasible at district level, but the main problem is that the company has not commercialised the test or applied for in vitro diagnostic registration and the assay cannot therefore be used for diagnostic purposes. Its use is restricted to research purposes only.

As discussed in Part 3 of this report, a simplified version of the test, designed by researchers at Northwestern University,[80] entered field trials in 2011 and it is anticipated that it will be available for diagnostic use in 2013.[81] The test will have to be suitable for ART-exposed infants for it to be useful in PMTCT programmes. In addition, other pipeline point-of-care tests discussed in Part 3 that amplify total nucleic acid (DNA plus RNA) from whole blood may also be useful for infant diagnosis.

In the meantime, HIV RNA testing may be used to diagnose treatment-naïve and treatment-unexposed infants,[82] for example, in the absence of PMTCT programmes or before the infant has started treatment. Viral load testing also has a use in the PMTCT, to determine the extent to which a mother is at risk of infecting her infant at birth and during breast-feeding.

PART 3

HOW TO ENSURE WIDER IMPLEMENTATION OF VIRAL LOAD: OVERCOMING TECHNICAL BARRIERS

PART 3:
HOW TO ENSURE WIDER IMPLEMENTATION OF VIRAL LOAD: OVERCOMING TECHNICAL BARRIERS

In 2010, a total of 1,175,000 viral load measurements were performed in the 66 countries reporting such data to WHO (specific countries are unknown)[83] – an average of 17,803 tests per country per year. By contrast, in South Africa, where routine viral load testing is supplied once a year to all patients on ART, a total of 1,386,130 tests were performed on one manufacturer's instrumentation alone in 2011.

One of the major reasons viral load monitoring has yet to be scaled up in resource-limited settings, especially in rural areas, is that current testing platforms used to measure HIV RNA are too complex and too laboratory-intensive. All commercial viral load tests are currently laboratory-based. These can range from medium to high technical complexity based on the level of automation or hands-on time and the need for precision pipetting. Most manufacturers offer only very large systems designed for central and reference laboratories. These tests are therefore not suited to district-level settings.

TABLE 2: COMMERCIALLY AVAILABLE VIRAL LOAD TESTS

	NUCLEIC ACID-BASED TECHNOLOGIES		
	Abbott	**Biocentric**	**bioMérieux**
	Abbott RealTime HIV-1 assay	**Generic HIV viral load assay**	**NucliSENS EasyQ® HIV-1 V2.0**
Assay Type	Real Time RT-qPCR with fluorescence detection	Real Time RT-qPCR with fluorescence detection	Real Time NASBA; isothermal signal amplification with chemiluminescent detection
Technological Set-Up	Fully automated or manual, closed system	Fully automated or manual, closed system	Fully automated, closed system
Extraction method/ sample preparation	Manual (mSample Preparation System) or automated (m24sp or m2000sp)	Manual (e.g. Qiagen spin column (QIAamp Viral RNA Mini Kit)) or automated (e.g. Nordiag Arrow)	Semi-manual (miniMAG) or automated (easyMAG)
Target	HIV-1 RNA pol	HIV-1 RNA LTR	HIV-1 RNA gag
HIV-1, HIV-2, Subtypes	HIV-1: Group M (A-H,CRF01_AE, CRF02_ AG), Group O, Group N	HIV-1: Group M (B and non-B subtypes including CRF)	HIV-1: Group M (A-J, CRF01_AE, CRF02_AG)
Linear Range	40 – 10,000,000 copies/ml	standard 300 – 50,000,000; ultrasensitive 40 – 50,000,000 copies/ml	25 – 10,000,000 copies/ml
Time to Result	5 – 7h	4h including RNA isolation	2.5 – 3h including extraction
Throughput	21 – 93 samples/run = 48 – 288 samples/d	min 1, max 96 samples/run = 192 samples/d	min 8, max 46 samples/run = 92 – 138 samples/d
Sample Type	Plasma, DBS (RUO)	Plasma, DBS (RUO)	Plasma, serum, DBS, any body fluid
Sample Volume	200µl – 1ml	standard 200µl – 500µl; ultrasensitive 400µl – 1,200µl	100µl, 500µl, 1ml of plasma; 2 DBS (2 x 50µl)
Controls	Internal control; Neg, low pos and high pos controls	None provided	Neg, low pos and high pos controls
Transport and Storage	refrigeration required	refrigeration required	refrigeration required
Equipment Required	m24sp or m2000sp (sample prep) plus m2000rt (amplification + detection)	Sample preparation kit/instrument plus any Real Time thermocycler (e.g. Bio-Rad C1000 thermocycler)	miniMAG or easyMAG (sample prep) plus EasyQ (amplification + detection) plus strip centrifuge
Cost of Equipment	m24sp: $90,000; m2000sp: $120,000; m2000rt: $50,000	Dependent on purchase choice of end-user (approximately $40,000)	miniMAG: $12,900; easyMAG: $114,100; easyQ: $57,400
Cost per Test	$15 – 70	$10 – 20	$15 – 27
Technical Skill	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes
Laboratory Set-Up	Specialised; 2-3 dedicated areas are required	Specialised; 2-3 dedicated areas are ideal but a single room protocol may be used (with a lab hood and thermal plate sealer)	Specialised; 2-3 dedicated areas are required
Storage Conditions	15-30°C: mSample Preparation System; -10°C: reagents, calibrators A+B and controls	Ideally down to -80°C although reagents may be stored at -20°C for short time periods	2-8°C: amplification reagents; 2-30°C: extraction reagents (buffers 1, 2 and lysis buffer); 2-8°C: buffer 3 and magnetic silica
Applicable Settings	Developed / highly resourced settings	Developing / low-medium resourced settings	Developed / highly resourced settings
Regulatory Approval	WHO PQ, CE-IVD, US-FDA-IVD, Canada-IVD, Japan-IVD, TGA (plasma)	Commercialised but currently RUO (WHO PQ and CE mark in process)	WHO PQ, CE-IVD (plasma and EDTA DBS)
Advantages	Use of DBS allows VL testing in remote areas; HIV DNA kit available for infant diagnosis	Use of DBS allows VL testing in remote areas; HIV DNA kit available for infant diagnosis; open access system allows purchasing from multiple companies	Use of DBS allows VL testing in remote areas; NASBA technique is RNA specific (no DNA amplification); is the only platform validated for use on DBS
Disadvantages	High resources required; co-amplifies DNA from whole blood	User input required to make best choices about which companies to purchase consumables from (Biocentric can implement the entire platform using Qiagen/Nordiag products plus Bio-Rad thermocycler); co-amplifies DNA from whole blood	High resources required; significant risk of contamination in high volume laboratories; HIV DNA testing for infant diagnosis is not possible

bDNA: branched DNA; **DBS:** dried blood spots; **ELISA:** enzyme-linked immunosorbent assay; **IVD:** in vitro diagnostic; **kPCR:** kinetic PCR; **LDC:** least-developed countries; **LTR:** long terminal repeat; **NASBA:** nucleic acid sequence based amplification; **PQ:** pre-qualified; **RT-qPCR:** reverse transcriptase quantitative polymerase chain reaction; **RUO:** research use only; **VL:** viral load.

Sources: 1. Product websites. **2.** Murtagh M. for UNITAID. HIV/AIDS Diagnostic Landscape, 2012. **3.** WHO Summary of Commercially Available Technologies for HIV Viral Load Testing and Early Infant Diagnosis. **4.** Stevens WS, Scott LE, Crowe SM. Quantifying HIV for monitoring antiretroviral therapy in resource-poor settings. The Journal of infectious diseases. 2010 Apr; 201 (Suppl 1): S16-26. **5.** Wang S, Xu F, Demirci U. Advances in developing HIV-1 viral load assays for resource-limited settings. Biotechnology advances. 2010; 28(6): 770-81.

	NUCLEIC ACID-BASED TECHNOLOGIES		
	Qiagen	Roche	Siemens
	artus® HI Virus-1 RG / QS-RGQ RT-PCR	COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) HIV-1 Test v2.0	VERSANT® HIV-1 RNA 3.0 Assay (bDNA)
Assay Type	Real Time RT-qPCR with fluorescence detection	Real Time RT-qPCR using detection by FRET	bDNA signal amplification using ELISA with chemiluminescent detection
Technological Set-Up	RG: Not fully automated, QS-RGQ: automated, closed system	Fully automated, closed system	Not fully automated
Extraction Method	RG: manual (QIAamp DSP Virus Kit) or QS-RGQ: automated	automated (docked and undocked options)	not applicable
Target	HIV-1 RNA LTR	HIV-1 RNA gag and LTR	HIV-1 RNA pol
HIV-1, HIV-2, Subtypes	HIV-1: Group M (A-H)	HIV-1: Group M (A-H), Group O	HIV-1: Group M (A-G)
Linear Range	RG: 60 – 50,000,000 copies/ml; QS-RGQ: 45 – 45,000,000	20 – 10,000,000 copies/ml	50 – 500,000 copies/ml
Time to Result	5 – 6h (per 24 reactions)	5-8h	22h
Throughput	67 samples/run	21 – 63 samples/run batch loading = 168 samples/d	12 – 192 samples/run
Sample Type	Plasma	Plasma, DBS (RUO)	Plasma
Sample Volume	RG: 500µl; QS-RGQ: 1,200µl	200µl – 1ml plasma, 1 DBS (60 – 70µl)	200µl – 1ml
Controls	Internal control; standard supplied at 4 different concentrations	Neg, low pos and high pos controls	Neg, low pos and high pos controls
Transport and Storage	refrigeration required	refrigeration required	refrigeration required
Equipment Required	QIAamp DSP Virus Kit or QIAsymphony SP/AS (sample prep) plus artus HI Virus-1 RG RT-PCR kit plus Rotor-Gene Q or Rotor-Gene 6000 or Rotor-Gene 3000 (amplification + detection)	COBAS AmpliPrep plus COBAS TaqMan 48 or 96	VERSANT 440 bDNA fully automated integrated analyzer plus VERSANT 440 factory refurbished centrifuge
Cost of Equipment	unknown	COBAS AmpliPrep: $80,000 – 150,000; COBAS TaqMan 48: $45,000 – 100,000; COBAS TaqMan 96: $80,000 – 150,000	$55,000
Cost per Test	unknown	LDC: $12 – 30; elsewhere: $16 – 90	$15 – 72
Technical Skill	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes
Laboratory Set-Up	Specialised; RG: 3, QS-RGQ: 2 dedicated areas are required	Specialised; 2-3 dedicated areas are required	Not specialised; single work area; deep-freezing required
Storage Conditions	down to -20°C	2-8°C	2-8°C: assay box A; down to -80°C: assay box B
Applicable Settings	Developed / highly resourced settings	Developed / highly resourced settings	Developed / highly resourced settings
Regulatory Approval	CE-IVD	WHO-PQ, CE-IVD, US-FDA-IVD, Canada-IVD, Japan-IVD (plasma)	CE-IVD, US-FDA-IVD
Advantages	None	Use of DBS allows VL testing in remote areas; HIV DNA kit available for infant diagnosis; single room technology	No nucleic acid extraction required; single room ELISA technology; high throughput
Disadvantages	High resources required; co-amplifies DNA from whole blood	High resources required; co-amplifies DNA from whole blood	Cannot use DBS; may be phased out by Siemens

bDNA: branched DNA; **DBS:** dried blood spots; **ELISA:** enzyme-linked immunosorbent assay; **IVD:** in vitro diagnostic; **kPCR:** kinetic PCR; **LDC:** least-developed countries; **LTR:** long terminal repeat; **NASBA:** nucleic acid sequence based amplification; **PQ:** pre-qualified; **RT-qPCR:** reverse transcriptase quantitative polymerase chain reaction; **RUO:** research use only; **VL:** viral load.

Continued overleaf ···

Table 2 continued

	NUCLEIC ACID-BASED TECHNOLOGIES	NON-NUCLEIC ACID-BASED TECHNOLOGIES	
	Siemens	Cavidi	PerkinElmer
	VERSANT HIV-1 RNA 1.0 Assay (kPCR)	ExaVir™ Load Version 3	Ultrasensitive p24 assay (not commercialised, RUO)
Assay Type	Real Time kPCR	ELISA of RT activity using colorimetric/ fluorimetric detection	Ultrasensitive, heat-denatured p24 ELISA with colorimetric / fluorimetric detection
Technological Set-Up	Fully automated, closed system	Not fully automated but closed system	Not fully automated but closed system
Extraction Method	Automated	Not applicable	Not applicable
Target	HIV-1 RNA pol	Retroviral reverse transcriptase activity	p24 (HIV core protein)
HIV-1, HIV-2, Subtypes	HIV-1: Group M (A-H, CRF01_AE, CRF02_AG), Group O	HIV-1 (all, subtype independent), HIV-2	HIV-1 (all), HIV-2 (some data)
Linear Range	30 – 11,000,000 copies/ml	~200 – 600,000 RNA copies/ml equivilent	~10,000 – 30,000 RNA copies/ml equivalent
Time to Result	5-6h	Colorimetric: 2.5d; fluorimetric: 1.5d	2.5 – 6h
Throughput	89 samples/run	30 samples/run = 30 – 60 samples/2d or <180/w	96 samples/run = 288 samples/d
Sample Type	Plasma, serum, DBS (RUO)	Plasma	Plasma, serum, cell culture supernatant
Sample Volume	500µl plasma and serum, 1 DBS (50 – 100µl)	1ml	50 – 450µl
Controls	Neg, low pos and high pos controls	Not provided (HIV neg and pos controls must be supplied in-house)	Standard supplied at 5 different concentrations
Transport and Storage	refrigeration required	refrigeration required	refrigeration required
Equipment Required	VERSANT kPCR Molecular System	Microplate reader with A405 filter plus incubator (33°C) plus freezer (-20°C plus end-over-end mixing table	Microplate reader plus incubator
Cost of Equipment	$166,000 – 222,000	$9,000 – 10,000	Dependent on ELISA plate reader purchased by end-user (approximately $7,000 – 9,000)
Cost per Test	$20 – 75	$13 – 15	$10 – 30
Technical Skill	Highly trained, precision pipetting required at low volumes	Low-moderately trained, precision pipetting required	Low-moderately trained, precision pipetting required
Laboratory Set-Up	Specialised; 2-3 dedicated areas are required; deep-freezing is required	Not specialised; single work area; freezing required	Not specialised
Storage Conditions	down to -30°C: kit IVDD box 1; down to -90°C: kit IVDD box 2; 15-30°C: sample prep reagents box 1; 2-8°C: sample prep reagents box 2	down to -20°C	2-8°C
Applicable Settings	Developed / highly resourced settings	Developing / low-medium resourced settings	Developing / low-medium resourced settings
Regulatory Approval	WHO PQ, CE-IVD (plasma)	CE marked	Not commercialised (RUO); No IVD registration
Advantages	Use of DBS allows VL testing in remote areas; HIV DNA kit available for infant diagnosis	Single room ELISA technology; subtype independent	Single room ELISA technology; subtype independent; can be used for infant diagnosis (although not registered as an IVD)
Disadvantages	High resources required; co-amplifies DNA from whole blood	Cannot use DBS; controls cannot be supplied; HIV DNA testing for infant diagnosis is not possible; very labour intensive	Not registered to be used as an IVD; separate external buffer required; cannot use DBS; cannot be used as a reliable diagnostic for treatment exposed infants; cannot be used as a treatment monitoring tool

bDNA: branched DNA; **DBS:** dried blood spots; **ELISA:** enzyme-linked immunosorbent assay; **IVD:** in vitro diagnostic; **kPCR:** kinetic PCR; **LDC:** least-developed countries; **LTR:** long terminal repeat; **NASBA:** nucleic acid sequence based amplification; **PQ:** pre-qualified; **RT-qPCR:** reverse transcriptase quantitative polymerase chain reaction; **RUO:** research use only; **VL:** viral load.

The Roche, Abbott, Siemens and bioMerieux tests have traditionally been restricted to central reference laboratories, although, given that the bioMerieux test is the only test validated for use with dried blood spots (DBS), it is more commonly used in settings where viral load testing has been reliant on long-distance DBS sample transport networks. Only two other viral load tests might be suitable for developing country settings outside of national laboratories: the Generic HIV Viral Load assay (produced by Biocentric), which, while still technically complex, has a small laboratory footprint; and the ExaVir Load test (produced by Cavidi), which is an ELISA-based assay that is not as prone to contamination problems compared to molecular assays and requires less stringent precision pipetting (although the sample preparation step is extremely labour-intensive and can be prone to contamination at this stage).[84] These two tests may be complementary in that the Biocentric assay may be used for high-throughput needs, whereas the Cavidi assay is more suited to medium throughput district-level laboratories. Importantly, these tests still need to be performed meticulously by trained technicians.

Laboratory capacity is a major issue in resource-limited settings, and the complexity of existing tests compounds this problem. Viral load testing requires dedicated, well-trained technicians and optimal internal and external quality assessment. A 2005–2007 survey by the WHO Africa Region on HIV/AIDS laboratory capacity[66] found that there is insufficient external quality assurance and human resources and a paucity of trained scientists to run laboratories.

GETTING VIRAL LOAD TO REMOTE SETTINGS: THE USE OF DBS FROM FINGERPRICK BLOOD

One way of providing testing closer to the patient is to place tests at the point of care. Another way is to send samples to the laboratory using DBS, and to provide a quick turnaround of results using, for example, mobile or electronic health technologies. Traditionally the preparation of DBS has required a nurse or phlebotomist to draw venous blood prior to the preparation of the DBS. In order to facilitate task shifting to less-qualified staff, MSF has validated the preparation of DBS from fingerprick capillary blood instead. This will allow sample collection from community health workers, which will relieve the workload burden for nurses at clinic level. MSF used the NucliSENS test (bioMérieux) as it is the only laboratory-based platform validated for use with this sample type (DBS prepared from venous blood).

265 patients were enrolled in Thyolo, Malawi, where MSF have been supporting an HIV programme since 1997. Patients had been on ART for at least 6 months and viral loads ranged from <20 to over 8 million copies/ml. The objective was to compare DBS prepared from 50µl of fingerprick blood to DBS prepared from the same amount of venous blood and plasma. Cut-offs for virological failure of both 1000 and 5000 copies/ml were chosen for comparative purposes. DBS were prepared by laboratory technicians. The results showed that DBS prepared from fingerprick blood performs as well as DBS prepared from venous blood when compared to plasma, with sensitivities around 90%, specificities close to 100% and high agreement compared to plasma at both cut-offs. Fingerprick DBS is therefore a viable alternative to plasma for measuring viral load.

The Malawi Ministry of Health has now approved this technique and task-shifting to nurses and lay-workers will now be validated in a second phase of the study, with fingerprick DBS compared to a reference standard. Going forward, MSF will continue to validate this technique in other countries and in other contexts.

Given that the DBS technique is currently the only means of sample transport over long distances and without the need for cold storage, it will be important for manufacturers of laboratory-based tests to validate their platforms for use with DBS.


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ENSURING QUALITY: THE RELIABILITY OF LABORATORY RESULTS AND OF DIAGNOSTIC TESTS

The issues of inadequate training and poor quality assurance have been identified as barriers to implementation of reliable diagnostic testing.[85] Nucleic acid amplification-based tests are prone to contamination as even a small amount of nucleic acid contamination can be amplified by molecular testing methods into a false positive result. This can be especially problematic in busy, high-throughput reference laboratories.

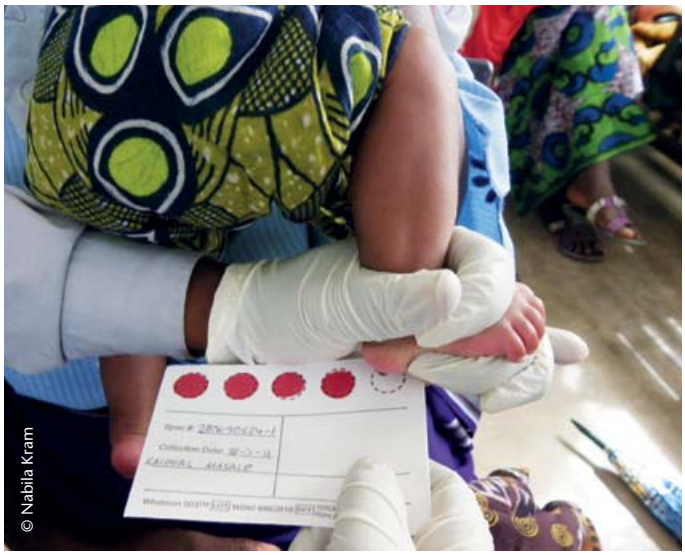
At present, MSF relies mainly on reference laboratories for the processing of HIV DNA and RNA assays. A recent study done by MSF in Southern Africa has shown that contamination at a well-recognised reference laboratory was severe and discordance between duplicate values unacceptable.[85] The clinical consequences of inaccurate results are concerning, with false positive results potentially leading to an incorrect diagnosis of virological failure and false negative results leading to undetected viremia.

The study concludes that every laboratory should be quality assessed and provides recommendations for identifying the suitability of an external laboratory for viral load testing.[85] It is also advised that, for the purpose of external quality control, samples be submitted to WHO-accredited reference laboratories or laboratories that have enrolled in the CDC's Proficiency Testing Programme.[86]

The quality certification of diagnostic products in resource-limited settings is currently not well regulated,[87] and most countries in Africa have no diagnostic regulatory authorities. As a consequence, US Food and Drug Administration approval or European Union CE marking (which rely, among other things, on ISO13485 manufacturing standards for commercialisation of products) are often used as a surrogate for quality assurance of tests, even though these products may not be suitable for resource-limited settings.[87] A WHO laboratory programme for the prequalification of products specifically suited to resource-limited settings was initiated in 2008. This process is much more thorough and hence slow moving, with only 11 products prequalified to date.[iii]

Concerns on quality will also be overcome by newer tools in the pipeline. If automated molecular testing were simplified enough for it to be feasible at the district or even the clinic level, it would alleviate demands on human resources, and reduce risks of contamination.

iii. http://www.who.int/diagnostics_laboratory/evaluations/PQ_list/en/index.html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TEST SPECIFICATIONS FOR RESOURCE-LIMITED SETTINGS?

Viral load testing is often required in remote settings without access to electricity or trained staff, and where transport of samples to reference laboratories can have a long turnaround time. Table 3 contains a wish list of specifications for both a simplified laboratory-based test and a point-of-care test.

These outcomes were based, in part, on the conclusions of a round-table expert meeting held in Paris in January 2005[54] and the findings of a comprehensive literature review. They address the simplification of tests that would be needed to enable their implementation at district level or rural laboratories and clinics.

TABLE 3: WISH LIST OF VIRAL LOAD TEST SPECIFICATIONS FOR RESOURCE-LIMITED SETTINGS

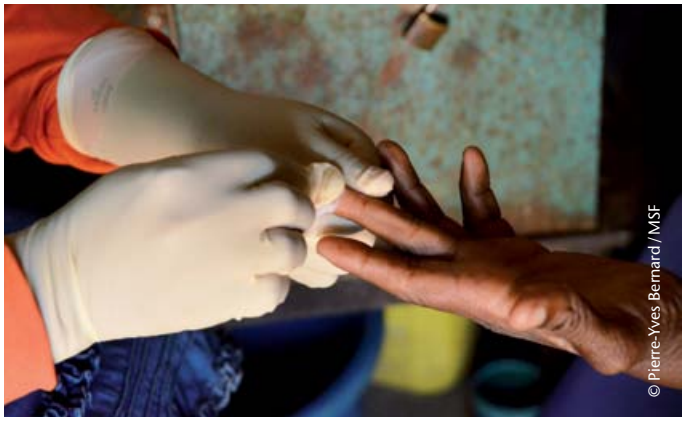
	Centralised, Laboratory-Based Test	Decentralised, Point-of-Care-Based Test
ASSAY CHARACTERISTIC		
Sample Collection Method	Plasma, DBS	Fingerstick, heelstick
Sample Volume	200–1000µl	≤100µl
Sample Preparation	Simple NA extraction method; no possibility of contamination	Simple electricity-free NA extraction method paired to simple POC VL test OR already part of automated POC VL test
Consumables per Result	Minimal; open access to consumables	Minimal e.g. 1 lancet, 1 capillary collection tube, 1 disposable cartridge
Reagent characteristics	Lyophilised reagents, no refrigeration necessary, stable to 40°C for ≥18m	Lyophilised reagents embedded on cartridge, no refrigeration necessary, stable to 40°C for ≥18m
Cost per Test	≤$10	≤$8
INSTRUMENT CHARACTERISTICS		
Power Requirements	AC and battery powered	AC, battery and solar powered (battery life should last ≥8 hours)
Characteristics	Open access to multiple different components and consumables; standardised operating procedure; basic laboratory required with single room technology and no risk of amplicon contamination	Single, closed system device; automated; benchtop/hand-held; easily portable; able to withstand extreme environmental conditions; able to function in a mobile, van-based clinic (i.e. able to withstand rigorous movement)
Cost of Instrument	All required instrumentation ≤$5,000 (e.g. centrifuge, plate sealer, thermocycler)	Single instrument ≤$1,000
PERFORMANCE		
Technician / healthcare worker hands-on time	≤1 hour	≤10 min
Time to Result	≤1 day	≤30 min
Analytic / Diagnostic Range	Quantitative; all HIV-1 subtypes; ≥50 viral copies/ml	Quantitative / semi-quantitative; all HIV-1 subtypes; ≥1,000 viral copies/ml (minimum threshold)
Training / Level of Skill	Medium level technical training	Minimal basic training (≤2 days); 10th grade education; no precision pipetting required
QUALITY		
Registered as an IVD	Minimum: WHO PQ; optional extra: CE marked and/or US-FDA approved	Minimum: WHO PQ; optional extra: CE marked and/or US-FDA approved

AC: alternating current; **DBS:** dried blood spot; **NA:** nucleic acid; **VL:** viral load; **POC:** point-of-care; **IVD:** in vitro diagnostic; **WHO PQ:** WHO Prequalification.
Source: Calmy A, Ford N, Hirschel B, Reynolds SJ, Lynen L, Goemaere E, et al. HIV Viral Load Monitoring in Resource-Limited Regions: Optional or Necessary? Clinical Infectious Diseases. 2007; 44(1): 128.

LOOKING TO THE PIPELINE

The validation and regulatory approval of simplified prototypes that are emerging from a pipeline of point of service testing options will greatly increase the ability to supply viral load testing in decentralised settings. There is a promising pipeline of products, the first commercialised prototypes of which should be available from 2013 onwards:[10]

- Liat nucleic acid analyzer (estimated release date: by 2013)
- Alere nucleic acid test (by 2014)
- WAVE80 EO-NAT HIV rapid RNA assay system (by 2014)
- SAMBA semi-quantitative test for viral load (by 2014)
- Northwestern Global Health Foundation PoC RT-PCR viral load (by 2015)
- Cavidi AMP (by 2015)
- GeneXpert with Xpert cartridge for viral load (after 2015)
- Lumora BART (after 2015)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- Micronics PanNAT diagnostics platform (after 2015)
- Advanced Liquid Logic viral load system (after 2015)
- BioHelix nucleic acid amplification platform (after 2015).

Table 4 provides a summary of available data on these tests.

Continued overleaf ···

Table 4 continued

TABLE 4: PIPELINE VIRAL LOAD TESTS[iv]

	IQuum	Northwestern University and Kellogg Global Health Initiative (Pr. David Kelso and Kara Palamountain)	
	Liat™ HIV Quant (released <2013)	p24 rapid lateral flow assay (released 2013)	POC RT-PCR Testing Platform (released 2014)
Assay Type	Real Time qPCR	Heat-denatured immunochromatographic test	Semi-quantitative PCR
Technological Set-Up	Portable; benchtop POC device; 1 sample per tube; fully automated and closed system; no batching capability; no maintenance (if damaged, on-site service/maintenance required)	Portable; disposable POC device; 1 sample tested sequentially; fully automated and closed system; no batching capability; no maintenance	Portable; disposable POC device; 1 sample per cartridge; fully automated and closed system; no batching capability
Target	HIV RNA	p24 (HIV core protein)	HIV RNA
HIV-1, HIV-2, Subtypes	Unknown	Subtype independent	Unknown
Linear Range	≥50 copies/ml	≥50 pg/ml or ≥42,500 RNA copies/ml equivalent	≥400 copies/ml
Time to Result	30-55 min (depending on limit of detection e.g. 500 copies/ml = 30 min)	30-40 min	~1h
Throughput	~8-15 samples/d	~16 samples/d	Unknown
Sample Type	Plasma, capillary blood	Capillary blood	Capillary blood
Sample Volume	Plasma 200µl, blood 10–50µl (fingerstick)	~80µl (heelstick)	~100µl (fingerstick or heelstick)
Controls	Multiple internal controls; tube cannot be retested	Internal control; lateral flow device cannot be retested	Unknown
Transport and Storage	Refrigeration required (4°C)	No refrigeration required	Unknown
Cost of Equipment	~$25,000 (maybe less for RLS)	Approx $400–700	Unknown
Cost per Test	TBD	Approx $7–15	Unknown
Technical Skill	Minimally trained	Minimally trained	Acquisition of a large volume of capilliary blood requires a specialised technique (massaging of finger, positioning of hand below heart)
Laboratory Set-Up	Laboratory not required; tube waste disposal necessary; AC or battery powered	Laboratory not required; test waste disposal necessary; heat block is battery powered (battery lasts 2y or ~100 tests)	Laboratory not required; test waste disposal necessary; battery powered
Applicable Settings	Decentralised facilities including mobile clinics, RLS	Decentralised facilities including mobile clinics, RLS	Decentralised facilitiess, RLS
Regulatory Approval	TBD	TBD	TBD
Advantages	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up)	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up); primary purpose is to be used for EID	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up)
Disadvantages	Low throughput; either phlebotomy or correct lancet blood draw is required; HR is required at clinic level	Low throughput; correct lancet blood draw is required; HR is required at clinic level; too insensitive to be used for viral load monitoring purposes	Low throughput; correct lancet blood draw is required; HR is required at clinic level; semi-quantitative

EID: early infant diagnosis; **HBV:** hepatitis B virus; **HCV:** hepatitis C virus; **HDA:** helicase dependent amplification; **NAAT:** nucleic acid amplification test; **POC:** point-of-care; **qPCR:** quantitative PCR; **RLS:** resource-limited settings; **TBD:** to be determined.

Sources: 1. Product websites. **2.** Personnal communications. **3.** Murtagh M. for UNITAID. HIV/AIDS Diagnostic Landscape, 2012.

iv. The tests are presented in order of estimated release date. Additional tests are estimated to be available from 2015 onwards. These include: Cavidi AMP, Cepheid GeneXpert viral load test, Lumora Bioluminescent Assay in Real-Time (BART), Micronics viral load test, Advanced Liquid Logic viral load test and Biohelix viral load lateral flow device.

	Alere	Wave 80	Cambridge University, Department of Haematology, Diagnostics Development Unit (Dr. Helen Lee)
	Alere NAT (released 2013)	**EOSCAPE-HIVTM System (released 2013)**	**SAMBA Analyser (released 2013)**
Assay Type	quantitative NAAT	quantitative NAAT	Semi-quantitative isothermal NAAT with visual detection (≥1,000 copies/ml)
Technological Set-Up	Portable; benchtop POC device; 1 sample per cartridge; fully automated and closed system; no batching capability; no maintenance (if damaged, swap out replacement rather than on-site repair)	Portable; benchtop POC device; 1 patient 1 test; fully automated and closed system	Portable; benchtop POC device; disposable cartridges; 1 sample per cartridge; no maintenance (if damaged swap out replacement)
Target	HIV RNA	HIV RNA	HIV RNA
HIV-1, HIV-2, Subtypes	HIV-1 (Groups M, N and O) and HIV-2	Unknown	HIV-1: Group M (A-K ,CRF01_AE, CRF02_AG), Group O, Group N
Linear Range	TBD	≥ a few hundred RNA copies/ml	Semi-quantitative threshold set at 1,000 copies/ml
Time to Result	30–60 min	50 min	90 min
Throughput	~10 samples/d	>50 samples/d (using 6-8 processing units and one analyser)	4 samples per run = 6 runs/6.5 h = 24 samples/d with 1 platform
Sample Type	Capillary blood	Plasma, capillary blood	Plasma
Sample Volume	~25µl (fingerstick)	Plasma unknown volume, blood 100µl (fingerstick)	Plasma 200µl
Controls	Internal control; cartridge cannot be retested	Internal control	Internal controls
Transport and Storage	No refrigeration required; sample stable for weeks within cartridge; freeze-dried reagents	No refrigeration required; cartridges stable at 37°C	No refrigeration required; cartridges stable at 37°C
Cost of Equipment	TBD	$10,000 for 1 analyser plus 2 processing units	TBD
Cost per Test	TBD	<$20	TBD
Technical Skill	Minimally trained	Minimally trained on device; acquisition of a large volume of capilliary blood requires a specialised technique (massaging of finger, positioning of hand below heart)	Minimally trained
Laboratory Set-Up	Laboratory not required; cartridge waste disposal necessary; rechargeable battery lasts for 8h	Laboratory not required; cartridge waste disposal necessary; rechargeable battery lasts for 8h (solar charging available)	Laboratory not required; test waste disposal necessary; AC or battery powered
Applicable Settings	Decentralised facilities including mobile clinics, RLS	Decentralised facilities including mobile clinics, RLS	Decentralised facilities, RLS
Regulatory Approval	TBD (Alere will apply for CE marking and FDA approval)	TBD	CE mark in progress; product approval received in Malawi
Advantages	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up)	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up); can be used for EID (qualitative test)	Technologically simple and POC (patient receives an immediate result and this allows for immediate clinical follow-up); can be used for EID (qualitative test) on whole blood
Disadvantages	Low throughput; correct lancet blood draw is required; HR is required at clinic level	Low throughput; either phlebotomy or correct lancet blood draw is required; HR is required at clinic level	Low throughput; either phlebotomy or correct lancet blood draw is required; semi-quantitative; HR is required at clinic level

EID: early infant diagnosis; **HBV:** hepatitis B virus; **HCV:** hepatitis C virus; **HDA:** helicase dependent amplification; **NAAT:** nucleic acid amplification test; **POC:** point-of-care; **qPCR:** quantitative PCR; **RLS:** resource-limited settings; **TBD:** to be determined.

All of these tests are designed to be low-cost and simple-to-use, with no or low electricity and maintenance requirements. Some are based on classical amplification methods while others are more innovative, using methods such as isothermal amplification, microarray technology or microfluidics.

As these tests have yet to become commercially available or validated for field use, it is not possible to give an accurate description of their technical accuracy or ease-of-use in resource-limited settings. However, they are expected to substantially increase the practicality of doing viral load tests in resource-limited settings.[88–90] Most of these prototypes are designed to be compatible with capillary "fingerprick" blood samples and have minimal electricity and maintenance requirements. Those with power requirements are designed to run off solar panels or a car battery, and they could be transported in mobile vans or on motorbikes. This will improve access to testing.

The majority of these new tools are designed to be point-of-care (PoC) tests. PoC testing presents considerable advantages for patients and providers: because diagnosis is performed at the point of service, at the clinic level, it eliminates the need for a return visit to collect results, as well as the risk of errors due to sample transportation or transcriptional mistakes.[54] The experience of the one existing device-based PoC laboratory monitoring test currently available for HIV patient monitoring is the PIMA CD4 Analyzer (produced by Alere). Although some technical caveats exists, this test has proven useful and has reduced loss to follow-up and delays between enrolment to ART initiation.[91] PoC tests would be particularly useful in the remotest settings, where access to laboratories is not an option.

However, PoC tests also come with caveats. Firstly, because they may be placed in environments with very high temperatures and levels of humidity, and possibly transported in mobile clinics, it remains to be seen whether or not they have been designed to be sufficiently environmentally robust. Secondly, because they work on a very small amount of blood, from a fingerprick, they may have reduced sensitivity compared to tests designed to work with a venous blood sample that can provide 1ml of plasma. Thirdly, the majority require an energy supply, necessitating reliable to access to electricity or another power source.

Fourthly, a major limitation to the rollout of decentralised testing capacity may be the shortage of human resources. By their very nature, these tests will have to be performed by clinic-level staff, such as nurses. Staff using the device will have to be adequately trained, not only on the performance of the test but also in quality assessment and control.[92] Assuming that these devices can be used at maximum capacity, most of these tests will take 30-60 minutes to perform, perhaps longer. This means that, if one device can process only one test at a time, it is unlikely to meet the throughput requirements of a busy clinic, especially as the number of patients on ART increase. For these reasons, both PoC as well as higher throughput district level laboratory-based tests are likely to needed.[93,94]

GETTING VIRAL LOAD TO REMOTE SETTINGS: MSF FIELD TRIALS OF THE SAMBA TEST

The SAMBA test (Simple AMplification Based Assay) was developed by the Diagnostics Development Unit at the University of Cambridge, with funding from the Wellcome Trust, the US National Institutes of Health and, more recently, The Children's Investment Fund Foundation. MSF participated in the prototype's field trial in Malawi and Uganda.

Most simplified tests provide a quantitative result but some, like the SAMBA, may be semi-quantitative, giving an "above or below" result at a specific threshold. In the case of the SAMBA viral load test, the threshold is set at 1000 copies/ml, corresponding to the threshold for treatment switch determined after several consensus meetings with HIV specialists.

The SAMBA test is a closed system where both sample preparation and amplification/detection reagents are pre-filled inside closed cartridges. This prevents contamination of the environment by amplicons (nucleic acid amplification products). Reagents are heat-stable and thus do not need cold chain transport or cold storage, which is a considerable advantage in many resource-limited settings. The test currently uses 200µL of plasma, therefore drawing venous blood is necessary, although manipulation is simple. A future version of the test will be compatible with whole blood. Results are obtained within 90 minutes. The SAMBA test can be implemented in district hospitals or health centres. In its current state of development, the test requires the use of two simple low-power electrical devices of small footprint, which can process four samples simultaneously, and could be transportable by motorbike. Staff training requirements are minimal.

Evaluations of the test were conducted successfully in 2011 in MSF sites at the Chiradzulu District Hospital, Malawi and the Arua District Hospital, Uganda, with high levels of concordance compared to gold standard real-time PCR viral load methods. Its routine use will now be implemented and further evaluated, operationally, in these two MSF sites and commercialisation is expected in 2013.

OTHER STRATEGIES TO OVERCOME COMPLEXITY

Aside from looking to the pipeline for simplified tests, a number of strategies can be pursued to ensure viral load monitoring can be implemented more widely in resource-limited settings.

- **Strategies to decentralise laboratories as much as possible:** Although it may not be possible to implement complex technologies at every healthcare facility, it may be possible to set up smaller laboratories equipped with testing platforms for the most important laboratory-based tests, for example, by refurbishing shipping containers.[95] Additionally, strategies that rely on point-of-care technology, such as mobile testing vans, can offer testing and monitoring services in the community.[96]

- **Strategies to pool viral load testing:** Pooled sample testing may be considered as a means to reduce the costs and throughput requirements of individual viral load testing. Pooling viral load testing involves the simultaneous assessment of five to ten samples from different patients in a single test, which is then run as a single sample. If the sample is positive, further tests will then need to be undertaken to identify positive results within the pool. As such, this method will only be useful in the testing of patients whose result is likely to be negative, and, as such, should be restricted to stable patients whose previous viral load result was undetectable. A study in Mexico found that pooled testing was able to decrease the number of tests run by the laboratory by one third, and allowed for the diagnosis of undetectable viral loads with >90% certainty (negative predictive value >90%).[97] A study in South Africa pre-selected samples for their pooled HIV RNA testing based on whether the patients had a low risk of viremia and had a previous viral load test result.[98] This resulted in a 30-60% reduction in individual tests required. The method should be further validated in resource-limited settings, especially those with demanding throughput requirements.

- **Strategies to improve simplified sample collection:** The primary method currently available for simplified transportation of blood samples from decentralised clinics to central laboratories is the "dried blood spot" (DBS): a defined volume of blood is pipetted onto filter paper, either from a venous blood sample or, even easier, from a fingerprick blood sample, after which it is preserved in a dessicated, air-tight bag that may be easily transported at ambient temperature.[78] DBS allows for the separation of sites for sampling from sites for testing.

  This inexpensive method has expanded access to viral load testing by making it possible to transport stabilised samples over great distances, even under harsh environmental conditions. The use of DBS has been technically validated for both viral load testing and genotyping.[40,99,100] WHO has suggested that the DBS technology needs to be "translated into quality-assured, widely available, standardised tools for health care providers".[2] This requires, in part, the standardisation of elution methods to allow for reproducible extraction yields.

  Although the use of DBS offers a novel, inexpensive and practical method of preserving viral nucleic acid for transportation to central laboratory facilities, there are a number of drawbacks. The small volume of sample collected (20µl – 200µl) greatly reduces the sensitivity of testing.[101] There is also a potential for false-positive viral load results due to pro-viral DNA contamination.[101–103] Finally, the lack of adequate storage conditions can lead to sample degradation, especially under extreme environmental conditions, such as high temperatures and humidity.[103]

  It would be useful to have a nucleic acid extraction method for point-of-care use in resource-limited settings that is more reliable than DBS. Exciting future prospects exist. One, for example, is the Fast Isolation of Nucleic Acid, a simple and inexpensive nucleic acid extraction method that takes two minutes to perform.[104] This extracted and preserved sample can then be sent to a central laboratory or paired to another point-of-care device, such as the one designed by PATH, where proof-of-concept for an inexpensive, electricity-free isothermal amplification of nucleic acid has been demonstrated.[105] Several isothermic (single temperature) prototypes are already available that show good performance compared to reference PCR methods.

PART 4

HOW TO ENSURE WIDER IMPLEMENTATION OF VIRAL LOAD: OVERCOMING MARKET BARRIERS

PART 4:
HOW TO ENSURE WIDER IMPLEMENTATION OF VIRAL LOAD: OVERCOMING MARKET BARRIERS

The cost of viral load testing is a significant barrier to its uptake. In Africa, costs can range between US$20-100 to run one test, depending on the technology, test, laboratory and elements included (such as reagents, instrumentation, and staff and running costs).[84,106] There have also been insufficient decreases in price over time. Historic trends show that prices have either remained the same or only marginally decreased, with a general increase in instrumentation costs, mainly because of the addition of automated sample extraction and preparation instruments.[10]

Table 5 lists some examples of the current test prices that MSF is paying.

Complexity of existing technologies has led to low demand for viral load testing, which in turn has limited competition and kept prices high. Furthermore, with no priority or political support to encourage viral load scale-up, the market continues to leave the development of more affordable technologies largely neglected. With such limited access to viral load, and a global viral load market size of 1.2 million tests per year, the market is not yet benefiting from volumes of scale that wider implementation would allow.

More broadly, the diagnostic market is fickle, with new tests having a short lifespan, as improved versions take their place, and regulatory approval is expensive, time-consuming and cumbersome. Instrument- or device-based tests also act to impede competition: once the initial instrument is purchased, in most cases only the manufacturer's reagents or cartridges can be purchased. In addition, once staff have been trained on a particular platform, there will be a reluctance to change to a new one.

At present there is no predictable approach to in-country registration of products, with most countries in Africa having no diagnostic regulatory authorities. Manufacturers may be able to import their products with no regulatory requirements whatsoever or, at the other extreme, have been asked to perform expensive trials on local populations before the Ministry of Health will provide permission for use. The African Society for Laboratory Medicine is trying to introduce a more standardised system to ensure regional collaboration and diagnostic quality without the need for redundant clinical trials. Surplus clinical trials delay the use of tests and increase costs for the manufacturer.

These factors create a high barrier to entry and a large disincentive for smaller, profit-based R&D companies. A 2009 analysis found that, globally, the nucleic acid testing market is currently dominated by one company, Roche (62%), with minimal competition from Siemens (17%), bioMerieux (11%) and Abbott (10%).[107]

TABLE 5: EXAMPLES OF PRICES PAID BY MSF

Laboratory	Country	Method	Price (local)	Price (US$)
Global Clinical and Viral Laboratory	South Africa	NucliSENS (BioMerieux)	ZAR 200	$26
National Health Laboratory Service	South Africa	NucliSENS (BioMerieux) and TaqMan (Roche)	ZAR 100 (varies)	$12 (can be up to $45)
Italian NGO Laboratory	Mozambique	unknown	-	$25
Private Laboratory	Zimbabwe	unknown	-	$90
Babina Diagnostics Laboratory	India	TaqMan (Roche)	INR 3700	$69

STRATEGIES TO REDUCE PRICES

A number of strategies could be pursued to reduce the price of viral load monitoring:

- **The impact of increased demand, and increased supply:** With eight million people currently receiving ART in developing countries, and a political ambition to scale up to 15 million by 2015, the potential market size is considerable. Although it is not realistic to expect that all patients on ART will have at least one viral load test per year by 2015, it is reasonable to expect at least a tripling of the current 1.2 million viral load tests performed annually in the 66 countries reporting data to WHO.[83] There is a large and growing market for inexpensive viral load monitoring in developing countries. In addition, the entry of simpler, lower-cost technologies could facilitate a scale-up of testing where not previously possible. This will increase both demand and supply, and, with economies of scale and the impact of competition between manufacturers, likely create downward pressure on price.

- **Increased price transparency:** Prices of diagnostics are often not transparent and vary significantly between countries and between buyers, through the effect of surcharges by distributors and variable discounts. Prices also vary based on quantities purchased, infrastructure, support required and special negotiations, with discounts usually provided for least-developed countries, but with middle-income countries generally excluded.[10,108] In some instances, the price differentials between tests may be unreasonable: for example, the Generic Viral Load Assay (Biocentric) is priced at $10-20 per test while the other molecular-based tests range between $20-100/test.[84,106] There is an urgent need for comprehensive and comparable publicly available pricing information.

- **Overcome barriers created by patents, know-how and trade secrets:** There is a plethora of overlapping patents, in the context of performing nucleic acid amplification,[109] that may hinder the development of generic products and may lead to high royalty payments that drive up prices. But, so far, in contrast to the significant attention drug patents have attracted, intellectual property (IP) barriers to affordable diagnostics have been largely ignored by policy makers, civil society and the media.

  The diagnostic patent landscape is poorly defined and patent barriers affecting the price and generic production of diagnostic and monitoring tests are largely unknown. Patent thickets, along with industry trade secrets and know-how, may make it difficult to analyse the patent landscape. More knowledge about the impact of patents is needed – a mapping of the IP landscape is currently being undertaken by UNITAID and this will shed more light on this issue. Once patent and other IP barriers have been identified, possible approaches could include, for example: support to patent challenges, patent oppositions, non-assert agreements, and pro-access licensing, such as royalty-free or low-royalty voluntary licences or compulsory licences.

- **Encourage the development of open platforms that can use other manufacturers' reagents:** While instrumentation often requires significant upfront investment, leasing or "reagent rental"[v] options can overcome this challenge.[110] However, the exclusivity of reagents presents more of a challenge in terms of running costs. Platforms capable of using quality-approved generic reagents offer a considerable advantage. Open testing systems stimulate competition and therefore drastically reduce the overall price of the test. While open platforms already exist in research settings, they need to be standardised, commercialised and quality assured in order to meet routine testing requirements.

  To date, only one generic testing platform (the Generic Viral Load and EID Assays produced by Biocentric) has been commercialised as a standard reagent kit. This assay, developed by a French academic institute in response to the inability of classical viral load assays to accurately measure non-B subtypes and recombinants in West African patients, was subsequently commercialised by Biocentric as a low-cost reagent kit. This kit is compatible with any real-time PCR machine and thus supports polyvalent instrumentation capabilities.

  Similarly, although the ExaVir Load assay by Cavidi is not a true open system in that the majority of the commodities still need to be purchased from the manufacturer (including the extraction platform), the test may be performed on any microplate reader (with an A405 filter). This means that the end-user does not have to purchase additional instrumentation to perform viral load testing if a PCR machine or microplate reader is already available in the laboratory.

- **Encourage the development of polyvalent platforms that diagnose different diseases:** Molecular technology is becoming increasingly important for the management of a range of infectious diseases of concern to resource-limited settings. Tuberculosis, trichomoniasis, chlamydia, gonorrhoea, herpes infections, chancroid (Haemophilus ducreyi), group B streptococcus, hepatitis B and C, human papilloma virus, and methicillin-resistant Staphylococcus aureus are examples. The ability to measure all of these diseases on the same platform would make the best use of transversal technologies and avoid laboratories having to invest in multiple instruments. WHO has acknowledged the need for multi-disease tools, recognising the absence of simple, high-throughput devices that could diagnose a range of diseases at point-of-care.[2] The caveat to this approach is that the manufacturers supplying the instrumentation must design their machine to be compatible with products from others, so as to retain commodity diversity and competition.

  A polyvalent platform could use different reagent kits or cartridges on the same instrument. For example, the FilmArray by Idaho provides a simultaneous measurement of 11 pathogens most likely to be causing upper respiratory tract infections. Another example of a polyvalent platform is the GeneXpert system by Cepheid. This modular platform, offered in different sizes, can take more than one cartridge at one time and Cepheid offers many different test cartridges so that one patient can be tested simultaneously for multiple diseases.

v. The benefits of reagent rental contracts are described here: http://www.frost.com/prod/servlet/market-insight-top.pag?docid=10985343html

Cepheid currently offers diagnostic cartridges for infectious diseases (e.g. C. difficile, methicillin-resistant Staphylococcus aureus, TB) and a number of cartridges are in the pipeline for measurement of viral load for HIV and hepatitis B and C, and human papillomavirus. Although this system is still relatively expensive and reliant on a stable electricity supply and air-conditioning, which limits feasibility in many settings, the concept of providing patients with a more comprehensive and immediate diagnosis has considerable appeal.

Table 6 provides an overview of the polyvalent capabilities of current nucleic acid amplification testing technologies.

TABLE 6: POLYVALENCY OF MOLECULAR NUCLEIC ACID AMPLIFICATION INSTRUMENTS

Range of tests able to be performed on a single instrumentation platform		
	Existing tests	**Potential polyvalency**
ExaVir Load (Cavidi)	HIV viral load	Any test dependent on the measurement of reverse transcriptase activity
Generic Viral Load Assay (Biocentric)	HIV-1 viral load and DNA*, M. tuberculosis (Hain Lifesciences)	Any test dependent on measurement by PCR
NucliSENS EasyQ HIV-1 v2.0 (bioMérieux)	Enterovirus, hMPV, HPV, Influenza (RUO), H5N1 (RUO), MRSA, RSV A&B, M. tuberculosis (RUO)	Any test dependent on measurement by NASBA
RealTime HIV-1 Assay (Abbott)	Hepatitis B&C, HPV, CT/NG, CMV, MRSA	Any test dependent on measurement by PCR
COBAS Ampliprep / COBAS Taqman HIV-1 Test v2.0 (Roche)	HIV-1 viral load and DNA*, Hepatitis B&C, M. tuberculosis, Clamydia, CMV viral load	Any test dependent on measurement by PCR
Artus® HI Virus-1 RG/QS-RGQ RT-PCR (Qiagen)	Hepatitis B, CMV, EBV, C. trachomatis, M. tuberculosis	Any test dependent on measurement by PCR

RUO: reseach use only. *HIV DNA is used for early infant diagnosis.

IS VIRAL LOAD MONITORING COST-EFFECTIVE?

A number of studies have sought to assess whether viral load monitoring at current prices were a cost-effective intervention.

One study found that, although clinical outcomes would be improved and transmission of resistant virus reduced following the introduction of viral load monitoring, this would come at the expense of not being able to afford to put newly infected patients on ART.[111] The authors concluded that a five-year delay was warranted in order to scale-up diagnosis and treatment of new patients and to await the availability of less expensive and better adapted virological testing options. In an accompanying editorial, others concur that a delay in viral load testing would better suit the needs of resource-limited countries.[69]

Another review of five studies conducting cost-effectiveness calculations on CD4 cell count and viral load monitoring in Africa concluded that, in general, CD4 count monitoring is cost-effective in most resource-limited settings but biannual HIV RNA monitoring, because it more often leads to a switch to more expensive second-line therapy, is only cost-effective in countries with higher per capita GDPs.[112,113]

In contrast, a Thai-based study found that, in most cases, viral load monitoring in children was cost-effective, and that even infrequent monitoring was clinically beneficial. The optimal monitoring frequency was once a year, after an initial test six months after ART initiation. Increased costs associated with viral load monitoring were mainly attributable to the increased use of more expensive second-line ART.[114]

As this market evolves, future cost effectiveness studies are likely to have divergent results. This is because existing studies do not reflect recent price reductions in both the costs of viral load tests and second-line ART, and do not take into account the likely reduction in cost in both as wider implementation of viral load occurs. More importantly, they do not compare immunological versus virological monitoring and it is therefore impossible to know how cost-effective it would be to use virological monitoring instead of immunological monitoring. As viral load is more relevant to treatment optimisation than CD4 count, cost-effectiveness studies comparing CD4 count versus viral load monitoring are urgently needed.

A recent modelling analysis using data from sub-Saharan Africa showed that both CD4 and viral load monitoring are cost-saving to a similar extent when used biannually, but CD4 monitoring more so compared to viral load monitoring when used annually.[115] The authors concluded that, although less cost-saving at 12-monthly testing intervals, viral load monitoring would still be preferred because of the additional benefits, such as, adherence support and the abrogation of drug resistance and viral transmission.

Future cost-effectiveness analyses should therefore consider more variables to give a fuller picture of the value of monitoring tools. There should also be a standardised calculation method for costs per test or drug, and an additional cost added for the initial implementation of new testing platforms.

Ultimately, viral load testing prices will have to come down, as well as the cost of second- and third-line ART, in order that viral load monitoring becomes more cost-effective.

CONCLUSIONS AND RECOMMENDATIONS

Access to routine viral load testing in resource-limited areas is urgently needed, as it is vital for long-term treatment optimisation, for the prevention of drug resistance, the preservation of current drug regimens, and ultimately improved patient outcomes.

The use of antiretroviral therapy without virological monitoring has been compared by some to "running with scissors" – everything appears to be simple and straightforward, until someone falls over.[116] In this sense, viral load is a way to minimize avoidable harm.

While clinical and immunological treatment monitoring can work, it is certainly not optimal, and we should not be denying patients or communities the benefits of virological monitoring that is the standard of care in the West. If we are to move to a new paradigm that seeks not just to increase CD4 cell count, but to reduce the amount of circulating virus to achieve both clinical and prevention goals, then urgent implementation of routine virological monitoring is imperative.

At today's prices of viral load tests and second-line ARVs, the introduction of viral load appears daunting. But, since technologies and prices of viral load are evolving fast, policy makers should not feel obliged to decide between funding new patient initiations or optimising and preserving treatment for those already on treatment. Both objectives can be attained and are mutually re-enforcing since diagnosing treatment failure means less circulating virus in communities and lower numbers of new infections.

Rapid introduction of viral load monitoring will require improving the effectiveness of diagnostics and monitoring tools, while using proven strategies to bring down the prices of both tests and treatments.

A phased approach should be pursued. This may include a number of strategies, such as: the use of dried blood spots to simplify transportation of samples to centralised laboratories; the use of pooled viral load testing to decrease sample throughput requirements; investigation of the possibility of reducing virological monitoring frequency in stable patients; the gradual replacement of CD4 with viral load monitoring, limiting use of CD4 to defining the time of treatment initiation; and the implementation of evidence-based algorithms to prioritise patients most urgently in need of virological monitoring.

Short- to medium-term objectives:

- The international HIV community must work together to ensure that viral load testing becomes the basic standard of care, and advocate for it to be implemented by all stakeholders.
- Donors should create incentives for more manufacturers to enter the market to increase competition and reduce the price per test. To date a lack of donor commitment has sent conflicting messages to the market place and has stifled R&D for simple tests designed for specific resource-limited settings.
- Strategies to reduce costs and make the current oligopolistic market more efficient and generate market competition should be explored. This includes facilitating new manufacturers to enter the market, creating greater price transparency, analysing and removing patent barriers where they exist, and giving preference to 'open systems', where feasible. Pooling demand from different programmes for joint price negotiation could also help to reduce costs.
- Operational research should assess the possibility of decreasing the frequency of testing, without compromising outcomes, and the possibility of replacing immunological monitoring with virological monitoring completely.
- The African Society for Laboratory Medicine, among other stakeholders, are aiming to transform the quality regulation of tests by implementing a standardised and predictable regulatory system that ensures the quality of tests without redundancy: They should be supported.

In the medium- to long-term:

- As low-cost pipeline technologies enter the market, donors must consider funding the field validation and implementation of these new prototypes for specific use in resource-limited settings, and support routine roll-out of new technologies.
- Manufacturers should design their prototypes to be compatible with reagents and consumables from other companies and for the diagnosis and monitoring of multiple diseases when possible.
- Operational research should be performed to provide an evidence base for the most feasible and most cost-effective tests.
- Future test development should consider screening for herald mutations. A screening test for mutations would help to confirm the type and extent of drug resistance to support treatment switching decisions so that clinicians can preserve first- and second-line drug regimens.

REFERENCES

1. World Health Organization: Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. 2010 Revision. 2010.
2. World Health Organization, UNAIDS: The Treatment 2.0 Framework for Action: Catalysing the Next Phase of Treatment, Care and Support. 2011.
3. Domingo G: Antiretroviral Treatment Monitoring Practices In Ten Countries: a Survey Performed by PATH Country Offices. 2010: 1–9.
4. Médecins Sans Frontières: MSF Activity Report. 2010: 1–108.
5. Keiser O, Chi B, Gsponer T, Boulle A, Orrell C, Phiri S, Maxwell N, Maskew M, Prozesky H, Fox MP, others: Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. AIDS 2011, 25: 1–10.
6. Lynen L, Van Griensven J, Elliott J: Monitoring for treatment failure in patients on first-line antiretroviral treatment in resource-constrained settings. Curr Opin HIV AIDS 2010, 5: 1–5.
7. Hamers RL, Wallis CL, Kityo C, Siwale M, Mandaliya K, Conradie F, Botes ME, Wellington M, Osibogun A, Sigaloff KCE, Nankya I, Schuurman R, Wit FW, Stevens WS, van Vugt M, de Wit TFR: HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis 2011, 11: 750–759.
8. Aghokeng AF, Kouanfack C, Laurent C, Ebong E, Atem-Tambe A, Butel C, Montavon C, Mpoudi-Ngole E, Delaporte E, Peeters M: Scale-up of antiretroviral treatment in sub-Saharan Africa is accompanied by increasing HIV-1 drug resistance mutations in drug-naive patients. AIDS 2011, 25: 2183–2188.
9. Gill VS, Lima VD, Zhang W, Wynhoven B, Yip B, Hogg RS, Montaner JSG, Harrigan PR: Improved Virological Outcomes in British Columbia Concomitant with Decreasing Incidence of HIV Type 1 Drug Resistance Detection. Clin Infect Dis 2010, 50: 98–105.
10. Murtagh M: UNITAID HIV/AIDS Diagnostic Landscape 2nd Edition. 2012.
11. Fitzgerland D: Survival, plasma HIV-1 RNA concentrations and drug resistance in HIV-1-infected Haitian adolescents and young adults on antiretrovirals. Bull World Health Organ 2008, 86: 970–977.
12. Usdin M, Guillerm M, Calmy A: Patient needs and point-of-care requirements for HIV load testing in resource-limited settings. J Infect Dis 2010, 201: S73–S77.
13. Rosen S, Sanne I, Collier A, Simon JL: Rationing antiretroviral therapy for HIV/AIDS in Africa: choices and consequences. PLoS Med 2005, 2: e303.
14. Bangsberg DR: Preventing HIV antiretroviral resistance through better monitoring of treatment adherence. J Infect Dis 2008, 197: S272–S278.
15. Ndembi N, Ndembi N, Goodall RL, Dunn DT, McCormick A, Burke A, Lyagoba F, Munderi P, Katundu P, Kityo C, Robertson V, Yirrell DL, Walker a S, Gibb DM, Gilks CF, Kaleebu P, Pillay D: Viral rebound and emergence of drug resistance in the absence of viral load testing: a randomized comparison between zidovudine-lamivudine plus Nevirapine and zidovudine-lamivudine plus Abacavir. J Infect Dis 2010, 201: 106–113.
16. Oyugi JH, Byakika-Tusiime J, Ragland K, Laeyendecker O, Mugerwa R, Kityo C, Mugyenyi P, Quinn TC, Bangsberg DR: Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. AIDS 2007, 21: 965–971.
17. Gilks CF, Crowley S, Ekpini R, Gove S, Perriens J, Souteyrand Y, Sutherland D, Vitoria M, Guerma T, De Cock K: The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet 2006, 368: 505–10.
18. Larson B a, Brennan A, McNamara L, Long L, Rosen S, Sanne I, Fox MP: Early loss to follow up after enrolment in pre-ART care at a large public clinic in Johannesburg, South Africa. Tropical medicine & international health: TM & IH 2010, 15 Suppl 1: 43–7.
19. Mills EJ, Nachega JB, Bangsberg DR, Singh S, Rachlis B, Wu P, Wilson K, Buchan I, Gill CJ, Cooper C: Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators. PLoS Med 2006, 3: e438.
20. Carrico AW, Bangsberg DR, Weiser SD, Chartier M, Dilworth SE, Riley ED: Psychiatric correlates of HAART utilization and viral load among HIV-positive impoverished persons. AIDS 2011, 25: 1113–1118.
21. Bangsberg DR, Deeks SG: Spending More to Save More: Interventions to Promote Adherence. Ann Intern Med 2010, 152: 54–56.
22. Nachega JB, Leisegang R, Bishai D, Nguyen H, Hislop M, Cleary S, Regensberg L, Maartens G: Association of antiretroviral therapy adherence and health care costs. Annals of internal medicine 2010, 152: 18.
23. Ware NC, Idoko J, Kaaya S, Biraro IA, Wyatt M a, Agbaji O, Chalamilla G, Bangsberg DR: Explaining adherence success in sub-Saharan Africa: an ethnographic study. PLoS medicine 2009, 6: e11.
24. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, Rachlis B, Wu P, Cooper C, Thabane L, Wilson K, Guyatt GH, Bangsberg DR: Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. J Am Med Assoc 2006, 296: 679–690.
25. Bärnighausen T, Chaiyachati K, Chimbindi N, Peoples A, Haberer J, Newell M-L: Interventions to increase antiretroviral adherence in sub-Saharan Africa: a systematic review of evaluation studies. The Lancet infectious diseases 2011, 11: 942–951.
26. Mills EJ, Lester R, Ford N: Adherence to antiretroviral therapy: supervision or support? Lancet Infect Dis 2012, 12: 97–98.
27. Montaner JSG, Lima VD, Barrios R, Yip B, Wood E, Kerr T, Shannon K, Harrigan PR, Hogg RS, Daly P, Kendall P: Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. Lancet 2010, 376: 532–539.
28. Donnell D, Baeten JM, Kiarie J, Thomas KK, Stevens W, Cohen CR, McIntyre J, Lingappa JR, Celum C: Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. Lancet 2010, 375: 2092–2098.
29. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JHS, others: Prevention of HIV-1 Infection with Early Antiretroviral Therapy. N Engl J Med 2011, 365: 493–505.
30. Quinn T, Wawer M, Sewankambo N: Viral Load and Heterosexual Transmission of Human Immunodeficiency Virus Type 1. N Engl J Med 2000, 342: 921–929.
31. Estill J, Aubriere C, Egger M, Johnson L, Wood R, Garone D, Gsponer T, Wandeler G, Boulle A, Davies M-A, Hallett T, Keiser O: Viral load monitoring of antiretroviral therapy, cohort viral load and HIV transmission in Southern Africa: A mathematical modelling analysis. AIDS 2012, 26: 1413.
32. Baeten JM, Kahle E, Lingappa JR, Coombs RW, Delany-Moretlwe S, Nakku-Joloba E, Mugo NR, Wald A, Corey L, Donnell D, Campbell MS, Mullins JI, Celum C: Genital HIV-1 RNA Predicts Risk of Heterosexual HIV-1 Transmission. Sci Transl Med 2011, 3: 77ra29.
33. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, Lima VD, Günthard HF, Gilks C, Pillay D: Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis 2009, 9: 409–417.
34. Rewari BB, Bachani D, Rajasekaran S, Deshpande A, Chan PL, Srikantiah P: Evaluating Patients for Second-Line Antiretroviral Therapy in India: The Role of Targeted Viral Load Testing. J Acquir Immune Defic Syndr 2010, 55: 610–614.
35. Simcock M, Sendi P, Ledergerber B, Keller T, Sch\"upbach J, Battegay M, G\"unthard HF, Swiss H: A longitudinal analysis of healthcare costs after treatment optimization following genotypic antiretroviral resistance testing: does resistance testing pay off? Antivir Ther 2006, 11: 305–314.
36. Wegner S a, Wallace MR, Aronson NE, Tasker S a, Blazes DL, Tamminga C, Fraser S, Dolan MJ, Stephan KT, Michael NL, Jagodzinski LL, Vahey MT, Gilcrest JL, Tracy L, Milazzo MJ, Murphy DJ, McKenna P, Hertogs K, Rinehart A, Larder B, Birx DL: Long-term efficacy of routine access to antiretroviral-resistance testing in HIV type 1-infected patients: results of the clinical efficacy of resistance testing trial. Clin Infect Dis 2004, 38: 723–730.
37. Weinstein M, Goldie S, Losina E, Cohen CJ, Baxter JD, Zhang H, Kimmel AD, Freedberg KA: Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. Ann Intern Med 2001, 134: 440–450.
38. Sendi P, Günthard HF, Simcock M, Ledergerber B, Schüpbach J, Battegay M: Cost-effectiveness of genotypic antiretroviral resistance testing in HIV-infected patients with treatment failure. PloS ONE 2007, 2: e173.
39. Chaix C, Grenier-Sennelier C, Clevenbergh P, Durant J, Schapiro J, Dellamonica P, Durand-Zaleski I: Economic Evaluation of Drug Resistance Genotyping for the Adaptation of Treatment in HIV-Infected Patients in the VIRADAPT Study. J Acquir Immune Defic Syndr 2000, 24: 227–231.

40. Yang C, McNulty A, Diallo K, Zhang J, Titanji B, Kassim S, Wadonda-Kabondo N, Aberle-Grasse J, Kibuka T, Ndumbe PM, Vedapuri S, Zhou Z, Chilima B, Nkengasong JN: Development and application of a broadly sensitive dried-blood-spot-based genotyping assay for global surveillance of HIV-1 drug resistance. J Clin Microbiol 2010, 48: 3158–3164.

41. Revell a D, Wang D, Harrigan R, Hamers RL, Wensing a MJ, Dewolf F, Nelson M, Geretti A-M, Larder B a: Modelling response to HIV therapy without a genotype: an argument for viral load monitoring in resource-limited settings. J Antimicrob Chemother 2010, 65: 605–607.

42. Cheung P, Wynhoven B, Harrigan P: Which HIV-1 drug resistance mutations are common in clinical practice? AIDS Rev 2004, 6: 107–116.

43. Harrigan PR, Hogg RS, Dong WWY, Yip B, Wynhoven B, Woodward J, Brumme CJ, Brumme ZL, Mo T, Alexander CS, Montaner JSG: Predictors of HIV drug-resistant mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. The Journal of infectious diseases 2005, 191: 339–47.

44. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, Carpenter S, Giddy J, Ross D, Holst H, others: Prevalence of HIV-1 Drug Resistance after Failure of a First Highly Active Antiretroviral Regimen in KwaZulu Natal, South Africa. Clin Infect Dis 2008, 46: 1589–1597.

45. Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R: HIV type-1 clade C resistance genotypes in treatment-naive patients and after first virological failure in a large community antiretroviral therapy programme. Antivir Ther 2009, 14: 523–531.

46. Singh A, Sunpath H, Green TN, Padayachi N, Hiramen K, Lie Y, Anton ED, Murphy R, Reeves JD, Kuritzkes DR, Ndung'u T: Drug Resistance and Viral Tropism in HIV-1 Subtype C-Infected Patients in KwaZulu-Natal, South Africa: Implications for Future Treatment Options. J Acquir Immune Defic Syndr 2011, 58: 233–240.

47. Bartlett J a, Shao JF: Successes, challenges, and limitations of current antiretroviral therapy in low-income and middle-income countries. Lancet Infect Dis 2009, 9: 637–649.

48. Hamers RL, Sigaloff KCE, Wensing AM, Wallis CL, Kityo C, Siwale M, Mandaliya K, Ive P, Botes ME, Wellington M, Osibogun A, Stevens WS, Rinke de Wit TF, Schuurman R: Patterns of HIV-1 Drug Resistance After First-Line Antiretroviral Therapy (ART) Failure in 6 Sub-Saharan African Countries: Implications for Second-Line ART Strategies. Clin Infect Dis 2012, 54: 1660–1669.

49. Jordan MR, Bennett DE, Wainberg M a, Havlir D, Hammer S, Yang C, Morris L, Peeters M, Wensing a M, Parkin N, Nachega JB, Phillips A, De Luca A, Geng E, Calmy A, Raizes E, Sandstrom P, Archibald CP, Perriëns J, McClure CM, Hong SY, McMahon JH, Dedes N, Sutherland D, Bertagnolio S: Update on World Health Organization HIV Drug Resistance Prevention and Assessment Strategy: 2004–2011. Clin Infect Dis 2012, 54: S245–S249.

50. Kelley KF, Caudwell E, Xueref S, Ha TH, Bertagnolio S: Are Countries Using Global Fund Support to Implement HIV Drug Resistance Surveillance? A Review of Funded HIV Grants. Clin Infect Dis 2012, 54: S250–S253.

51. Kerschberger B, Boulle AM, Kranzer K, Hilderbrand K, Schomaker M, Coetzee D, Goemaere E, Ford N, Cutsem GV: Viral load at 3 months after initiation of antiretroviral therapy is associated with better virological and treatment outcomes than at 6 months. In International AIDS Conference abstract MOPE144. 2011, 12.

52. Ford N, Darder M, Spelman T, Maclean E, Mills E, Boulle A: Early Adherence to Antiretroviral Medication as a Predictor of Long-Term HIV Virological Suppression: Five-Year Follow Up of an Observational Cohort. PLoS One 2010, 5: e10460.

53. Oyomopito R, Lee MP, Phanuphak P, Lim PL, Ditangco R, Zhou J, Sirisanthana T, Chen YM a, Pujari S, Kumarasamy N, Sungkanuparph S, Lee CKC, Kamarulzaman A, Oka S, Zhang FJ, Mean CV, Merati T, Tau G, Smith J, Li PCK: Measures of site resourcing predict virologic suppression, immunologic response and HIV disease progression following highly active antiretroviral therapy (HAART) in the TREAT Asia HIV Observational Database (TAHOD). HIV Med 2010, 11: 519–29.

54. Calmy A, Ford N, Hirschel B, Reynolds SJ, Lynen L, Goemaere E, de la Vega FG, Perrin L, Rodriguez W: HIV Viral Load Monitoring in Resource-Limited Regions: Optional or Necessary? Clin Infect Dis 2007, 44: 128–134.

55. Wilson D, Keiluhu a K, Kogrum S, Reid T, Seriratana N, Ford N, Kyawkyaw M, Talangsri P, Taochalee N: HIV-1 viral load monitoring: an opportunity to reinforce treatment adherence in a resource-limited setting in Thailand. Trans R Soc Trop Med Hyg 2009, 103: 601–606.

56. Sigaloff KCE, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, Botes ME, Mandaliya K, Wellington M, Osibogun A, Stevens WS, van Vugt M, Rinke de Wit TF: Unnecessary Antiretroviral Treatment Switches and Accumulation of HIV Resistance Mutations; Two Arguments for Viral Load Monitoring in Africa. J Acquir Immune Defic Syndr 2011, 58: 23–31.

57. Keiser O, Tweya H, Boulle A, Braitstein P, Schecter M, Brinkhof MWG, Dabis F, Tuboi S, Sprinz E, Pujades-Rodriguez M, Calmy A, Kumarasamy N, Nash D, Jahn A, MacPhail P, Lüthy R, Wood R, Egger M: Switching to second-line antiretroviral therapy in resource-limited settings: comparison of programmes with and without viral load monitoring. AIDS 2009, 23: 1867–1874.

58. Kanapathipillai R, McGuire M, Mogha R, Szumilin E, Heinzelmann A, Pujades-Rodriguez M: Benefit of viral load testing for confirmation of immunological failure in HIV patients treated in rural Malawi. Trop Med Int Health 2011, 16: 1495–1500.

59. Moore DM, Awor A, Downing R, Kaplan J, Montaner JSG, Hancock J, Were W, Mermin J: CD4+ T-cell count monitoring does not accurately identify HIV-infected adults with virologic failure receiving antiretroviral therapy. J Acquir Immune Defic Syndr 2008, 49: 477–484.

60. Davies M-A, Boulle A, Eley B, Moultrie H, Technau K, Rabie H, van Cutsem G, Giddy J, Wood R, Egger M, Keiser O: Accuracy of immunological criteria for identifying virological failure in children on antiretroviral therapy – The IeDEA Southern Africa Collaboration. Trop Med Int Health 2011, 16: 1367–1371.

61. Rawizza HE, Chaplin B, Meloni ST, Eisen G, Rao T, Sankalé J-L, Dieng-Sarr A, Agbaji O, Onwujekwe DI, Gashau W, Nkado R, Ekong E, Okonkwo P, Murphy RL, Kanki PJ: Immunologic Criteria Are Poor Predictors of Virologic Outcome: Implications for HIV Treatment Monitoring in Resource-Limited Settings. Clin Infect Dis 2011, 53: 1283–1290.

62. Oliveira R, Krauss M, Essama-Bibi S, Hofer C, Robert Harris D, Tiraboschi A, de Souza R, Marques H, Succi R, Abreu T, Della Negra M, Hazra R, Mofenson LM, Siberry GK: Viral load predicts new world health organization stage 3 and 4 events in HIV-infected children receiving highly active antiretroviral therapy, independent of CD4 T lymphocyte value. Clin Infect Dis 2010, 51: 1325–1333.

63. Brown ER, Otieno P, Mbori-Ngacha D a, Farquhar C, Obimbo EM, Nduati R, Overbaugh J, John-Stewart GC: Comparison of CD4 cell count, viral load, and other markers for the prediction of mortality among HIV-1-infected Kenyan pregnant women. J Infect Dis 2009, 199: 1292–1300.

64. Reekie J, Gatell J, Yust I, Bakowska E, Rakhmanova A, Losso M, Krasnov M, Francioli P, Kowalska JD, Mocroft A: Fatal and non-fatal AIDS and non-AIDS events in HIV-1 positive individuals with high CD4 counts according to viral load strata. AIDS 2011, 25: 2259–2268.

65. Maldonado F, Biot M, Roman F, Masquelier C, Anapenge M, Bastos R, Chuquela HC, Arendt V, Schmit JC, Zachariah R: Viraemia and HIV-1 drug resistance mutations among patients receiving antiretroviral treatment in Mozambique. Trans R Soc Trop Med Hyg 2009, 103: 607–612.

66. World Health Organization Regional Office for Africa: HIV and AIDS Laboratory Capacity. Where are we? Overview of laboratory capacity in Africa 2005 – 2007. 2010: 1–96.

67. World Health Organization, UNAIDS: Joint WHO/UNAIDS Annual Consultation with Pharmaceutical Companies. Global Forecasts of Antiretroviral Demand 2012 – 2013. 2011: 1–23.

68. Chang LW, Harris J, Humphreys E: Optimal monitoring strategies for guiding when to switch first-line antiretroviral therapy regimens for treatment failure in adults and adolescents living with HIV in low-resource settings. Cochrane database of systematic reviews 2011: 1–48.

69. Korenromp EL, Fakoya A, Viisainen K: Scaling-up antiretroviral treatment in resource-poor countries: prioritization and choices. AIDS 2011, 25: 857–859.

70. World Health Organization: Scaling up antiretroviral therapy in resource-limited settings: Treatment guidelines for a public health approach. 2003: 1–67.

71. Orrell C, Harling G, Lawn SD, Kaplan R, McNally M, Bekker L-G, Wood R: Conservation of first-line antiretroviral treatment regimen where therapeutic options are limited. Antivir Ther 2007, 12: 83–88.

72. Rouet F, Ménan H, Viljoen J, Ngo-Giang-Huong N, Mandaliya K, Valéa D, Lien TX, Danaviah S, Rousset D, Ganon A, Nerrienet E: In-house HIV-1 RNA real-time RT-PCR assays: principle, available tests and usefulness in developing countries. Expert Rev Mol Diagn 2008, 8: 635–650.

73. Lynen L, An S, Koole O, Thai S, Ros S, De Munter P, Sculier D, Arnould L, Fransen K, Menten J, Boelaert M, Van den Ende J, Colebunders R: An algorithm to optimize viral load testing in HIV-positive patients with suspected first-line antiretroviral therapy failure in Cambodia. Journal of acquired immune deficiency syndromes (1999) 2009, 52: 40–8.

74. Havlir DV, Bassett R, Levitan D, Gilbert P, Tebas P, Collier AC, Hirsch MS, Ignacio C, Condra J, G\"unthard HF, others: Prevalence and Predictive Value of Intermittent Viremia With Combination HIV Therapy. JAMA: the journal of the American Medical Association 2001, 286: 171.

75. Lee PK, Kieffer TL, Siliciano RF, Nettles RE: HIV-1 viral load blips are of limited clinical significance. J Antimicrob Chemother 2006, 57: 803–805.

76. Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, Cofrancesco J, Gallant JE, Quinn TC, Jackson B, others: Intermittent HIV-1 Viremia (Blips) and Drug Resistance in Patients Receiving HAART. J Am Med Assoc 2005, 293: 817–829.

77. World Health Organization: Antiretroviral therapy for HIV infection in infants and children: Towards Universal Access. Recommendations for a public health approach. 2010 Revision. 2010.

78. Johannessen A, Trøseid M, Calmy A: Dried blood spots can expand access to virological monitoring of HIV treatment in resource-limited settings. J Antimicrob Chemother 2009, 64: 1126–1129.

79. Schüpbach J, Böni J, Bisset LR, Tomasik Z, Fischer M, Günthard HF, Ledergerber B, Opravil M: HIV-1 p24 antigen is a significant inverse correlate of CD4 T-cell change in patients with suppressed viremia under long-term antiretroviral therapy. Journal of acquired immune deficiency syndromes (1999) 2003, 33: 292–9.

80. Parpia ZA, Elghanian R, Nabatiyan A, Hardie DR, Kelso DM: p24 antigen rapid test for diagnosis of acute pediatric HIV infection. Journal of acquired immune deficiency syndromes 2010, 55: 413–9.

81. Nabatiyan A, Baumann M a, Parpia Z, Kelso D: A lateral flow-based ultra-sensitive p24 HIV assay utilizing fluorescent microparticles. Journal of acquired immune deficiency syndromes (1999) 2010, 53: 55–61.

82. Kfutwah AKW, Tejiokem MC, Ateba FN, Ndongo JA, Penda IC, Ngoupo PAT, Tchendjou P, Chewa G, Boisier P, Rouzioux C, Warszawski J, Faye A: Seronegativation in Early Treated HIV-Infected Infants: Frequency and Potential Implications on Care and Follow-up in a Resource-Limited Country. JAIDS 2011, 58: e43–e46.

83. Souteyrand Y: Towards Universal Access? Scaling up priority HIV/AIDS interventions in the health sector. In WHO Meeting. 2011.

84. Wang S, Xu F, Demirci U: Advances in developing HIV-1 viral load assays for resource-limited settings. Biotechnol Adv 2010, 28: 770–781.

85. Greig J, du Cros P, Klarkowski D, Mills C, Jørgensen S, Harrigan PR, O'Brien DP: Viral load testing in a resource-limited setting: quality control is critical. J Int AIDS Soc 2011, 14: 23.

86. Gershy-Damet G-M, Rotz P, Cross D, Belabbes EH, Cham F, Ndihokubwayo J-B, Fine G, Zeh C, Njukeng P a, Mboup S, Sesse DE, Messele T, Birx DL, Nkengasong JN: The World Health Organization African region laboratory accreditation process: improving the quality of laboratory systems in the African region. Am J Clin Pathol 2010, 134: 393–400.

87. World Health Organization: Medical device regulations. Global overview and guiding principles. 2003, 24: 1–43.

88. Sukapirom K, Onlamoon N, Thepthai C, Polsrila K, Tassaneetrithep B, Pattanapanyasat K: Performance evaluation of the Alere PIMA CD4 test for monitoring HIV-infected individuals in resource-constrained settings. J Acquir Immune Defic Syndr 2011, 58: 141–147.

89. Diaw PA, Daneau G, Coly AA, Ndiaye BP, Wade D, Camara M, Mboup S, Kestens L, Dieye TN: Multisite Evaluation of a Point-of-Care Instrument for CD4 + T-Cell Enumeration Using Venous and Finger-Prick Blood: The PIMA CD4. J Acquir Immune Defic Syndr 2011, 58: 103–111.

90. Jani IV, Sitoe NE, Chongo PL, Alfai ER, Quevedo JI, Tobaiwa O, Lehe JD, Peter TF: Accurate CD4 T-cell enumeration and antiretroviral drug toxicity monitoring in primary healthcare clinics using point-of-care testing. AIDS 2011, 25: 807–812.

91. Jani IV, Sitoe NE, Alfai ER, Chongo PL, Quevedo JI, Rocha BM, Lehe JD, Peter TF: Effect of point-of-care CD4 cell count tests on retention of patients and rates of antiretroviral therapy initiation in primary health clinics: an observational cohort study. Lancet 2011, 6736: 1–8.

92. Palamountain KM, Baker J, Cowan EP, Essajee S, Mazzola LT, Metzler M, Schito ML, Stevens WS, Young GJ, Domingo GJ: Perspectives on Introduction and Implementation of New Point-of-Care Diagnostic Tests. J Infect Dis 2012, 205: S181–S190.

93. Kassaye SG, Katzenstein D: The point of point-of-care testing. Lancet 2011, 378: 1532–3.

94. Schito ML, Peter TF, Cavanaugh S, Piatek AS, Young GJ, Alexander H, Coggin W, Domingo GJ, Ellenberger D, Ermantraut E, Jani IV, Katamba A, Palamountain KM, Essajee S, Dowdy DW: Opportunities and Challenges for Cost-Efficient Implementation of New Point-of-Care Diagnostics for HIV and Tuberculosis. J Infect Dis 2012, 205: S169–S180.

95. Grant RM: Research in situ. Nat Methods 2007, 4: 887–890.

96. Kranzer K, Bekker L-g, Schaik NV: Community health care workers in South Africa are at increased risk for tuberculosis. South Afr Med J 2010, 100: 224–226.

97. Tilghman MW, Guerena DD, Licea A, Pérez-Santiago J, Richman DD, May S, Smith DM: Pooled nucleic acid testing to detect antiretroviral treatment failure in Mexico. J Acquir Immune Defic Syndr 2011, 56: e70–e74.

98. Van Zyl GU, Preiser W, Potschka S, Lundershausen a. T, Haubrich R, Smith D: Pooling Strategies to Reduce the Cost of HIV-1 RNA Load Monitoring in a Resource-Limited Setting. Clin Infect Dis 2010, 52: 264–270.

99. Reigadas S, Schrive MH, Aurillac-Lavignolle V, Fleury HJ: Quantitation of HIV-1 RNA in dried blood and plasma spots. J Virol Methods 2009, 161: 177–180.

100. Rottinghaus E, Ugbena R, Diallo K: Dried Blood Spot Specimens Are a Suitable Alternative Sample Type for HIV-1 Viral Load Measurement and Drug Resistance Genotyping in Patients Receiving First-Line Antiretroviral Therapy. Clin Infect Dis 2012, 54: 1187–1195.

101. Hamers R, Smit P, Stevens W, Schuurman R: Dried fluid spots for HIV type-1 viral load and resistance genotyping: a systematic review. Antivir Ther 2009, 14: 619–629.

102. Johannessen A: Quantification of HIV-1 RNA on dried blood spots. AIDS 2010, 24: 475.

103. Monleau M, Montavon C, Laurent C, Segondy M, Montes B, Delaporte E, Boillot F, Peeters M: Evaluation of different RNA extraction methods and storage conditions of dried plasma or blood spots for human immunodeficiency virus type 1 RNA quantification and PCR amplification for drug resistance testing. J Clin Microbiol 2009, 47: 1107–1118.

104. Jangam SR, Yamada DH, McFall SM, Kelso DM: Rapid, point-of-care extraction of human immunodeficiency virus type 1 proviral DNA from whole blood for detection by real-time PCR. J Clin Microbiol 2009, 47: 2363–2368.

105. Labarre P, Hawkins KR, Gerlach J, Wilmoth J, Beddoe A, Singleton J, Boyle D, Weigl B: A Simple, Inexpensive Device for Nucleic Acid Amplification without Electricity-Toward Instrument-Free Molecular Diagnostics in Low-Resource Settings. PloS one 2011, 6: e19738.

106. Stevens WS, Scott LE, Crowe SM: Quantifying HIV for monitoring antiretroviral therapy in resource-poor settings. J Infect Dis 2010, 201: S16–S26.

107. Largen M: Nucleic Acid Testing: A Vehicle for Advancing Personalized Medicine. 2009.

108. UNICEF, UNAIDS, WHO, MSF: Sources and prices of selected medicines and diagnostics for people living with HIV. 2005: 1–78.

109. Norviel V, Wilson, Sonsini G& R: PCR IP Landscape. In qPCR Symposium. 2009.

110. Elbeik T, Dalessandro R, Loftus R a, Beringer S: HIV-1 and HCV viral load cost models for bDNA: 440 Molecular System versus real-time PCR AmpliPrep/TaqMan test. Expert review of molecular diagnostics 2007, 7: 723–53.

111. Phillips AN, Pillay D, Garnett G, Bennett D, Vitoria M, Cambiano V, Lundgren J: Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings. AIDS 2011, 25: 843–850.

112. Walensky RP, Ciaranello AL, Park J-E, Freedberg K a: Cost-effectiveness of laboratory monitoring in sub-Saharan Africa: a review of the current literatur. Clin Infect Dis 2010, 51: 85–92.

113. Kimmel AD, Weinstein MC, Anglaret X, Goldie SJ, Losina E, Yazdanpanah Y, Messou E, Cotich KL, Walensky RP, Freedberg K a: Laboratory monitoring to guide switching antiretroviral therapy in resource-limited settings: clinical benefits and cost-effectiveness. J Acquir Immune Defic Syndr 2010, 54: 258–268.

114. Schneider K, Puthanakit T, Kerr S, Law MG, Cooper D a, Donovan B, Phanuphak N, Sirisanthana V, Ananworanich J, Ohata J, Wilson DP: Economic evaluation of monitoring virologic responses to antiretroviral therapy in HIV-infected children in resource-limited settings. AIDS 2011, 25: 1143–1151.

115. Hamers RL, Sawyer a W, Tuohy M, Stevens WS, de Wit TFR, Hill AM: Cost-effectiveness of laboratory monitoring for management of HIV treatment in sub-Saharan Africa: a model-based analysis. AIDS 2012, Epub.

116. Smith DM, Schooley RT: Running with scissors: using antiretroviral therapy without monitoring viral load. Clin Infect Dis 2008, 46: 1598–1600.

117. Meynard J-L, Vray M, Morand-Joubert L, Race E, Descamps D, Peytavin G, Matheron S, Lamotte C, Guiramand S, Costagliola D, Brun-Vézinet F, Clavel F, Girard P-M: Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. AIDS 2002, 16: 727–736.

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