Developments in CD4 and Viral Load Monitoring in Resource-Limited Settings

Christopher F. Rowley[1,2,3]

[1]Division of Infectious Diseases, Beth Israel Deaconess Medical Center; [2]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts; and [3]Botswana-Harvard AIDS Institute, Gaborone

CD4 counts and human immunodeficiency virus (HIV) load testing are essential components of HIV care, and making these tests available in resource-limited settings is critical to the roll-out of HIV treatment globally. Until recently, the evidence supporting the importance of laboratory monitoring in resource-limited settings was lacking, but there is now a consensus emerging that testing should become routine to ensure the longevity of treatment programs. Low-cost, point-of-care testing offers the potential to fill this role as it potentially improves all aspects of HIV care, ranging from the diagnosis and staging of HIV infection in both infants and adults to monitoring for treatment failure once antiretroviral therapy has been initiated. It is imperative for low-cost solutions to become a reality, but it is equally imperative that close scrutiny be given to each new device that hits the market to ensure they perform optimally in all settings.

Keywords. HIV; resource-limited; treatment monitoring; CD4; viral load.

CD4 count and human immunodeficiency virus (HIV) load tests have long been a part of the routine monitoring of HIV infection, but in resource-limited settings the ideal strategies for employing these tests are unknown [1, 2]. This review will focus on the importance of monitoring CD4 count and viral load in resource-limited settings and the current laboratory challenges encountered in these regions and will review new instruments currently available or scheduled to be available within 12 months with the potential to make point-of-care laboratory testing a viable, economical option.

World Health Organization (WHO) guidelines updated in 2013 recommend CD4 testing at the time of HIV diagnosis, with initiation of antiretroviral treatment (ART) if the CD4 count is <500 cells/mm^3 [3]. CD4 count testing is also performed every 6 months while on treatment to monitor immunologic response to ART, with additional CD4 count testing at time of treatment failure.

HIV load testing is not recommended at the time of HIV diagnosis but is recommended 6 months into ART treatment and every 12 months thereafter to detect treatment failure. Plasma viral load >1000 copies/mL on 2 consecutive measurements at least 3 months apart, in the setting of adherence counseling, indicates treatment failure and the need to change to second-line ART. The guidelines stress that both CD4 and viral load testing should be performed only if resources permit, and treatment should not be withheld if laboratory capabilities are not available.

BENEFITS OF DIFFERENT MONITORING STRATEGIES

The need for close laboratory monitoring of HIV care in resource-limited settings was evaluated in 2 randomized trials: the Development of Antiretroviral Therapy in Africa (DART) trial [4] and the Home-Based AIDS Care Project (HBAC) [5]. In the DART trial, >3000 individuals were followed over 5 years with excellent survival rates in both the clinical monitoring–only arm and the laboratory testing arm: survival rates were 87%

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Correspondence: Christopher F. Rowley, MD MPH, Division of Infectious Diseases, Lowry Bldg, Ste GB, 110 Francis St, Boston, MA 02215 (crowley1@bidmc.harvard.edu).

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and 90%, respectively. There was more disease progression from year 2 to year 5 in the group monitored clinically, suggesting a role for CD4 count monitoring. The HBAC study in rural Uganda randomized 1094 patients to a clinical arm, an arm in which the CD4 count was followed routinely, or an arm in which both CD4 count and viral load were followed. The rates of new AIDS-defining events or death were higher in the clinical arm than in either the CD4 arm or the dual monitoring arm, but there was no significant difference between the latter 2 arms.

VIRAL LOAD MONITORING

Viral load monitoring is potentially very important in resource-limited settings for 2 reasons. The first reason is to prevent changing of the first-line ART to a suboptimal, expensive, second-line therapy when unnecessary [6–8]. A study from Kenya evaluated 149 patients who were suspected to have failed immunologically, and these patients had both CD4 testing as well as viral load testing performed [7]. If CD4 monitoring alone was used, about half would have switched ART despite actually having undetectable HIV viral loads (<400 copies/mL).

The second reason is to prevent individuals from languishing on failing therapies, which allows drug resistance to develop [9]. In a multicenter study in southern Africa, individuals who were being followed clinically and immunologically were compared with a group that additionally received viral load testing [10]. Close to 50% of patients in the group without viral load testing were changed to second-line ART unnecessarily, similar to the Kenya study, but many individuals with clinical failure did actually have virologic failure. When genotypes were performed on these 183 samples, 80% had at least 1 resistance mutation, with 40% having cross-resistance to the nucleoside reverse transcriptase inhibitors.

COST EFFECTIVENESS OF ROUTINE MONITORING

The cost effectiveness of laboratory monitoring was studied in both the DART and HBAC trials [5, 11]. Data from the DART trial suggested that CD4 monitoring would not be cost effective unless it was <$3.70/sample. However, if there was a corresponding decrease in the cost of second-line ART, then CD4 count testing could become cost effective. The HBAC trial reached a somewhat different conclusion, suggesting that the addition of routine CD4 count testing to clinical monitoring was cost effective, but there was no additional economic benefit of viral load monitoring. Several modeling studies suggest that it is cost effective to monitor routinely for both CD4 count and viral load [12–14]. Although not in complete agreement, data from both clinical and modeling studies suggest that if second-line ART is available at low cost, then routine monitoring could be economical. However, given finite resources, widespread ART availability should be the imperative, and its importance may outweigh the need for routine laboratory testing [13].

LABORATORY DEVICES

In many places around the world, the gold standard for CD4 count testing is large platform machines, including the BD FACSCalibur or the Beckman Coulter Cytomic FC 500 platform, which process 200–500 samples/day. This equipment has an initial purchasing cost of $75 000–100 000 [15], but the equipment's high throughput capacity can dramatically reduce costs to $3–$7 per sample. However, considerable resources are required, including daily maintenance, a steady supply of reagents, well-trained staff, a reliable electricity supply, and ongoing quality assurance evaluations. Because these platforms are located mostly in cities or at centralized reference laboratories, the transportation of samples to these facilities is challenging and the relaying of the results to the patient-care site can be equally difficult.

These issues can be mitigated by the use of smaller machines that comprise the same flow cytometry technology but require less resources, allowing them to be used closer to the point of care. The FACSCount by BD Biosciences is commonly used in much of the resource-limited world for CD4 testing. It allows the processing of 50–80 samples/day. The machine costs approximately $30 000, with an individual test costing $3–$10 [15].

The difficulties encountered in implementing this technology to a point-of-care testing site were noted in a study detailing the roll-out of a point-of-care CD4 count machine (Guava Easy CD4 Assay) in Burkina Faso and Zimbabwe [16]. The investigators encountered many challenges, including inadequate access to repair services during instrument breakdown, an inability to maintain the supply of reagents, and high technician turnover, particularly in Zimbabwe where there were particularly challenging sociopolitical circumstances. The end result was that 60% of individuals in Burkina Faso and 92% of individuals in Zimbabwe had only 1 CD4 value obtained, receiving an initial CD4 count but failing to return for follow-up. This falls drastically short of the testing required as part of an antiretroviral treatment program.

UNITAID (a global health organization enhancing diagnostics and treatment for HIV, malaria, and tuberculosis) and the WHO announced the HIV Treatment 2.0 Guidelines in 2011, of which point-of-care diagnostics are a core component [17]. A 2009 study from Zambia [18] shows the potential impact of point-of-care testing. At that time, the Zambian national program allowed women who were pregnant to start combination ART if their CD4 count was <350 cells/mm^3 even if they were clinically asymptomatic. More than 14 000 pregnant

women tested positive for HIV, yet only 2500 of them were tested for CD4 count, an 83% drop-off. This highlights the fact that the clinical setting is where rapid testing is most needed, testing needs to take place, shortening the time from diagnosis to staging of HIV disease.

CD4 POINT-OF-CARE TESTING

Currently, 3 devices that perform point-of-care testing for CD4 count are commercially available. The first device is the Partec CyFlow miniPOC [15]. This desktop device provides both CD4 percentages and absolute CD4 counts. It costs approximately $10 000, and it is estimated that each CD4 count would cost $4. It is based on the same flow cytometry technology used in the company's larger device, but currently there are no peer-reviewed studies about its performance.

PointCare Now, presently called HumaCount CD4 Now, was introduced into >160 countries in April 2012 [15]. The device costs approximately $25 000, and 1 CD4 count will be close to $10. The results are available within 8–10 minutes and include absolute CD4 count, CD4 percentage, white blood cell count and differential, and hemoglobin concentration. At the time it was released, there were no peer-reviewed studies of this device, but in August 2012 a study [19] suggested the device significantly overestimated CD4 counts. When a threshold of 350 cells/mm^3 for ART initiation was used, the machine only identified 47% of the patients who should have started treatment. When a threshold of 200 cells/mm^3 was used, it only identified 39% who would have qualified for ART.

The third currently available point-of-care CD4 device is the Alere Pima Analyser. This device calculates an absolute CD4 count but not CD4 percentages. A user could perform up to 20 tests/day. It has an initial cost of $6000–12 000, and an individual sample costs $6–12 [15]. Many studies have suggested it performs quite well [20–22], but one suggested that it may underestimate CD4 counts compared with the gold standard [23]. It demonstrated good concordance in pregnant women compared with gold standard testing, an important consideration for the antenatal clinic setting [22].

One group's experience using the Pima Analyser in several different settings found discrepant results in a rural field site compared with urban sites, with the rural field site having a positive bias of 105 cells/mm^3 [24]. The need for reinforced education about the proper technique for capillary sampling was deemed paramount. These investigators concluded that the best results occurred when a venous blood draw was performed, with the blood subsequently transferred to a capillary, resulting in an additional step that required more expertise and exposure to risk than previously anticipated. This group and another [25] also report on the unanticipated costs associated with implementation of the device, including costs for lancets, quality control consumables, instrument maintenance, and nursing/phlebotomy.

An observational study using the Pima device was the first to show evidence of the impact of the point-of-care instrument on healthcare delivery [26]. This study compared outcomes of 494 patients who presented to care before the introduction of this test to 437 individuals who presented to care after this device was introduced. The proportion lost to follow-up decreased from 57% to 21% after the test was used. The proportion of enrolled patients initiating ART increased from 12% to 22%, with the time from enrollment to the start of treatment decreasing by 28 days.

Several new devices are in the pipeline, and those close to market are highlighted. The Daktari CD4 Counter is anticipated to be commercially available within the next year [15]. This device is designed to be sturdy and could be brought to the most remote sites, with a CD4 result available in 8 minutes. An alternative is MBio Diagnostics' point-of-care CD4 test [27], which is made entirely of plastic. Blood incubates on a cartridge for 20 minutes, allowing for samples to be done in parallel. A fixative is added after incubation, and the samples are placed serially into the test reader for 3 minutes to obtain an absolute CD4 count. It is estimated that 10 samples can be completed per hour. Two other testing modalities, the device-free Zyomyx CD4 test, which consists of a cartridge that is spun for 10 minutes and yields an absolute CD4 count, and the BD FACSPresto, which is capable of providing a CD4 count, CD4 percentage, and hemoglobin on 1 disposable cartridge, are anticipated to be launched before the end of 2013 [28].

Lastly, a semiquantitative test, the Visitect CD4, is nearing commercial availability [28]. This device measures proteins rather than T cells directly. The readout is on a strip, and a line becomes visible that will signify that the CD4 count is >350 cells/mm^3. A reader device accompanies the test strip so the result in not dependent on operator interpretation.

VIRAL LOAD MONITORING DEVICES

The technical challenges of CD4 count testing are less difficult to overcome than those associated with viral load testing. Typical viral load testing requires additional infrastructure, including continuous power, clean running water, air conditioning, multiple clean rooms to eliminate contamination, and the facility to centrifuge samples while maintaining the cold chain [29]. Transport is again of high importance, as is the need to ensure a strict adherence to quality assurance.

Medecins sans Frontieres shared their experience in a sub–Saharan research laboratory [30], demonstrating some of the challenges. In one region, 53% of patients who were treatment-naive achieved virologic suppression at 6 months, whereas close to 85% of patients were suppressed in other regions. This

discordance resulted in an investigation of the laboratory, and >50% of the viral load results were deemed invalid compared with a reference laboratory. More than 2500 test results were discarded as a consequence. A move to another well-trained laboratory was still met with challenges. More than 300 of the first 614 patients tested did not receive their test results a month later because there were delays in procuring the assay kits and lack of in-country technical support, demonstrating that implementation, even in well-respected laboratories, may be difficult.

One attempt to improve viral load testing in resource-limited settings is the Simple AMplification-Based Assay, which was developed at Cambridge University and is nearing commercial availability [31]. This assay is a desktop nucleic acid amplification and detection device, with the process taking place in a closed cartridge and providing both semiquantitative and qualitative results in one hour. The semiquantitative test will be used for treatment monitoring, with the ability to determine if a sample has >1000 or <1000 copies/mL of HIV RNA. The qualitative test has a threshold of 100 copies/mL and will be used for early infant diagnosis.

Another device, the LiatAnalyser, is a nucleic acid testing platform that has already been approved for influenza H1N1 and influenza A and B, and this represents another fully automated HIV load device [32]. If the user chooses the setting of 500–1000 copies/mL, a result will be available in 30 minutes; it will take 1 hour if the threshold of detection is 50 copies/mL, as is done for early infant diagnosis. This fully contained desktop instrument performs all aspects of sample handling from RNA isolation to amplification and detection.

Other devices in the pipeline include the NAT system and the EOSCAPE-HIV HIV Rapid RNA Assay System, which hopefully will be released some time during 2013, allowing for robust field comparisons of the different devices.

OTHER STRATEGIES FOR VIRAL LOAD TESTING

Dried blood spots have been shown to be useful for HIV diagnosis and resistance testing [33–36] and could be applicable to resource-limited settings. A recent study from India demonstrated how this technique could be used for treatment monitoring [37]. The sensitivity of the testing of dried blood spots was 62%, 80%, and 100% when the viral load thresholds were <1000 copies/mL, 1000–3000 copies/mL, and >3000 copies/mL. This approach eliminates the need for specialized sample collection and maintenance of the cold chain.

Lastly, pooling of samples has been successful in testing large numbers of people for acute HIV infection at far less cost than individual viral load testing [38, 39]. It may be used to monitor for treatment failure as well [40–42]. By pooling, which creates 1 large sample pool comprised of smaller sample pools, many samples have viral load testing performed at once. If the pool tests negative, all of the samples comprising the larger pool are assumed to have suppressed virus; if it is positive, the larger pool is divided into its component smaller pools, and retested. Ultimately, it is possible to determine which individual samples had a detectable viral load with fewer total viral load tests performed. In a study from Mexico, pooling would have saved >33% of viral load tests required in a cohort of 700 samples compared with individual monitoring [41], whereas other studies show 31%–60% few viral load tests by pooling methods [40, 43].

BEYOND LOGISTICAL CHALLENGES

As HIV diagnostic testing has moved to remote testing centers and antenatal clinics, it has vastly increased the opportunity for individuals to learn their HIV status and for the prevention of infant infections as women learn their HIV status and receive prophylaxis to decrease the vertical transmission of HIV [44–46]. Viral load testing on dried blood spots for early infant diagnosis has also been rolled out, but with mixed success because the tests are not performed in real time and the delay in obtaining test results can mean individuals are lost to follow-up.

The current push towards inexpensive point-of-care options for CD4 and viral load testing will mitigate the logistical challenges, bridging the divide between diagnosis and staging of HIV, but testing alone will not solve the issue of linkage to care. Only 62% of patients attended their referral appointment within 8 weeks after HIV diagnosis in a recent South African study when CD4 counts were provided by point-of-care testing, compared with 47% who did so when the CD4 count was not provided [47]. In Mozambique, only 22% of individuals who had their HIV infection staged by point-of-care testing ultimately started ART [26]. Point-of-care testing is a requisite next step, but the WHO Treatment Guidelines 2.0 correctly recognize that this is only part of a multifaceted approach to provide universal access to HIV treatment [17].

In addition, cost-effectiveness analyses will continue to be essential with all of the individual point-of-care tests being devised to support their implementation. These analyses allow for the thoughtful interplay of policy and healthcare with issues such as the determination of what viral load threshold allows for the most treatment failures to be detected without unnecessary changes to expensive, second-line therapy [48, 49]. The rapid pace of the technical advances needs to be juxtaposed with the ability to provide appropriate treatment for the greatest number of individuals infected with HIV.

CONCLUSIONS

The field is rapidly evolving, and more data will emerge as new testing modalities are introduced. In 2012, Medecins sans Frontieres and UNITAID announced they will be starting a $20

million project to implement point-of care CD4 testing and viral load testing to demonstrate the feasibility and cost-effectiveness of "radically decentralizing viral load monitoring and CD4 testing from top-level reference laboratories down to district level facilities" [50]. It will be imperative to the long term success of treatment programs globally that these efforts succeed.

Notes

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References

1. Arnedo M, Alonso E, Eisenberg N, et al. Monitoring HIV viral load in resource limited settings: still a matter of debate? PLoS One **2012**; 7: e47391.
2. Ford N, Roberts T, Calmy A. Viral load monitoring in resource-limited settings: a medical and public health priority. AIDS **2012**; 26:1719–20.
3. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treatment and Preventing HIV Infection. Geneva, Switzerland: WHO, **2013**.
4. Mermin J, Ekwaru JP, Were W, et al. Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial. BMJ **2011**; 343: d6792.
5. Kahn JG, Marseille E, Moore D, et al. CD4 cell count and viral load monitoring in patients undergoing antiretroviral therapy in Uganda: cost effectiveness study. BMJ **2011**; 343:d6884.
6. Rawizza HE, Chaplin B, Meloni ST, et al. Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings. Clin Infect Dis **2011**; 53: 1283–90.
7. Kantor R, Diero L, Delong A, et al. Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis **2009**; 49:454–62.
8. Westley BP, DeLong AK, Tray CS, et al. Prediction of treatment failure using 2010 World Health Organization Guidelines is associated with high misclassification rates and drug resistance among HIV-infected Cambodian children. Clin Infect Dis **2012**; 55:432–40.
9. Group A-LoISKeiser O, Tweya H, Boulle A, et al. Switching to second-line antiretroviral therapy in resource-limited settings: comparison of programmes with and without viral load monitoring. AIDS **2009**; 23:1867–74.
10. Sigaloff KC, Hamers RL, Wallis CL, et al. 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.
11. Medina Lara A, Kigozi J, Amurwon J, et al. Cost effectiveness analysis of clinically driven versus routine laboratory monitoring of antiretroviral therapy in Uganda and Zimbabwe. PloS One **2012**; 7:e33672.
12. Kimmel AD, Weinstein MC, Anglaret X, et al. Laboratory monitoring to guide switching antiretroviral therapy in resource-limited settings: clinical benefits and cost-effectiveness. J Acquir Immune Defic Syndr **2010**; 54:258–68.
13. Braithwaite RS, Nucifora KA, Yiannoutsos CT, et al. Alternative antiretroviral monitoring strategies for HIV-infected patients in east Africa: opportunities to save more lives? J Int AIDS Soc **2011**; 14:38.
14. Walensky RP, Ciaranello AL, Park JE, Freedberg KA. Cost-effectiveness of laboratory monitoring in sub-Saharan Africa: a review of the current literature. Clin Infect Dis **2010**; 51:85–92.
15. UNITAID. HIV/AIDS diagnostic technology landscape. 2nd ed. **2012**. Available at: http://www.unitaid.eu/images/marketdynamics/publications/UNITAID-HIV_Diagnostics_Landscape-2nd_edition.pdf. Accessed 7 November 2013.
16. Thairu L, Katzenstein D, Israelski D. Operational challenges in delivering CD4 diagnostics in sub-Saharan Africa. AIDS Care **2011**; 23:814–21.
17. World Health Organization. The treatment 2.0 framework for action: catalysing the next phase of treatment, care and support. **2011**. Available at: http://whqlibdoc.who.int/publications/2011/9789241501934_eng.pdf. Accessed 7 November 2013.
18. Mandala J, Torpey K, Kasonde P, et al. Prevention of mother-to-child transmission of HIV in Zambia: implementing efficacious ARV regimens in primary health centers. BMC Public Health **2009**; 9:314.
19. Bergeron M, Daneau G, Ding T, et al. Performance of the PointCare NOW system for CD4 counting in HIV patients based on five independent evaluations. PloS One **2012**; 7:e41166.
20. 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**; 58:141–7.
21. Herbert S, Edwards S, Carrick G, et al. Evaluation of PIMA point-of-care CD4 testing in a large UK HIV service. Sex Transm Infect **2012**; 88:413–7.
22. Mnyani CN, McIntyre JA, Myer L. The reliability of point-of-care CD4 testing in identifying HIV-infected pregnant women eligible for antiretroviral therapy. J Acquir Immune Defic Syndr **2012**; 60:260–4.
23. Manabe YC, Wang Y, Elbireer A, Auerbach B, Castelnuovo B. Evaluation of portable point-of-care CD4 counter with high sensitivity for detecting patients eligible for antiretroviral therapy. PloS One **2012**; 7: e34319.
24. Glencross DK, Coetzee LM, Faal M, et al. Performance evaluation of the Pima point-of-care CD4 analyser using capillary blood sampling in field tests in South Africa. J Int AIDS Soc **2012**; 15:3.
25. Larson B, Schnippel K, Ndibongo B, Long L, Fox MP, Rosen S. How to estimate the cost of point-of-care CD4 testing in program settings: an example using the Alere Pima Analyzer in South Africa. PLoS One **2012**; 7:e35444.
26. Jani IV, Sitoe NE, Alfai ER, et al. 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**; 378:1572–9.
27. Logan C, Givens M, Ives JT, et al. Performance evaluation of the MBio Diagnostics point-of-care CD4 counter. J Immunol Methods **2013**; 387:107–13.
28. UNITAID. HIV/AIDS Diagnostics Technology Landscape, Semi-annual Update, **2012**. Available at: http://www.unitaid.eu/images/marketdynamics/publications/UNITAID_2012_Semi-annual_Update_HIV_Diagnostics_Technology_Landscape.pdf.pdf. Accessed 7 November 2013.
29. Stevens WS, Marshall TM. Challenges in implementing HIV load testing in South Africa. J Infect Dis **2010**; 201 (Suppl 1):S78–84.
30. Greig J, du Cros P, Klarkowski D, et al. Viral load testing in a resource-limited setting: quality control is critical. J Int AIDS Soc **2011**; 14:23.
31. Lee HH, Dineva MA, Chua YL, Ritchie AV, Ushiro-Lumb I, Wisniewski CA. Simple amplification-based assay: a nucleic acid-based point-of-care platform for HIV-1 testing. J Infect Dis **2010**; 201 (Suppl 1): S65–72.

32. Tanriverdi S, Chen L, Chen S. A rapid and automated sample-to-result HIV load test for near-patient application. J Infect Dis **2010**; 201 (Suppl 1):S52–8.
33. Okonji JA, Basavaraju SV, Mwangi J, et al. Comparison of HIV-1 detection in plasma specimens and dried blood spots using the Roche COBAS Ampliscreen HIV-1 test in Kisumu, Kenya. J Virol Methods **2012**; 179:21–5.
34. Viljoen J, Gampini S, Danaviah S, et al. Dried blood spot HIV-1 RNA quantification using open real-time systems in South Africa and Burkina Faso. J Acquir Immune Defic Syndr **2010**; 55:290–8.
35. Johannessen A, Garrido C, Zahonero N, Naman E, de Mendoza C. HIV-1 drug resistance testing from dried blood spots collected in rural Tanzania using the ViroSeq HIV-1 Genotyping System. J Antimicrob Chemother **2011**; 66:260–4.
36. Rottinghaus EK, Ugbena R, Diallo K, et al. 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**; 54:1187–95.
37. Vidya M, Saravanan S, Rifkin S, et al. Dried blood spots versus plasma for the quantitation of HIV-1 RNA using a real-time PCR, m2000rt assay. J Virol Methods **2012**; 181:177–81.
38. Pilcher CD, Fiscus SA, Nguyen TQ, et al. Detection of acute infections during HIV testing in North Carolina. N Engl J Med **2005**; 352:1873–83.
39. Sullivan TJ, Patel P, Hutchinson A, Ethridge SF, Parker MM. Evaluation of pooling strategies for acute HIV-1 infection screening using nucleic acid amplification testing. J Clin Microbiol **2011**; 49:3667–8.
40. van Zyl GU, Preiser W, Potschka S, Lundershausen AT, Haubrich R, Smith D. Pooling strategies to reduce the cost of HIV-1 RNA load monitoring in a resource-limited setting. Clin Infect Dis **2011**; 52:264–70.
41. Tilghman MW, Guerena DD, Licea A, et al. Pooled nucleic acid testing to detect antiretroviral treatment failure in Mexico. J Acquir Immune Defic Syndr **2011**; 56:e70–4.
42. May S, Gamst A, Haubrich R, Benson C, Smith DM. Pooled nucleic acid testing to identify antiretroviral treatment failure during HIV infection. J Acquir Immune Defic Syndr **2010**; 53:194–201.
43. Smith DM, May SJ, Perez-Santiago J, et al. The use of pooled viral load testing to identify antiretroviral treatment failure. AIDS **2009**; 23:2151–8.
44. Creek TL, Alwano MG, Molosiwa RR, et al. Botswana's Tebelopele voluntary HIV counseling and testing network: use and client risk factors for HIV infection, 2000–2004. J Acquir Immune Defic Syndr **2006**; 43:210–8.
45. Marum E, Taegtmeyer M, Parekh B, et al. "What took you so long?" The impact of PEPFAR on the expansion of HIV testing and counseling services in Africa. J Acquir Immune Defic Syndr **2012**; 60 (Suppl 3): S63–9.
46. Heller T, Kunthea S, Bunthoeun E, et al. Point-of-care HIV testing at antenatal care and maternity sites: experience in Battambang Province, Cambodia. Int J STD AIDS **2011**; 22:742–7.
47. Larson BA, Schnippel K, Ndibongo B, et al. Rapid point-of-care CD4 testing at mobile HIV testing sites to increase linkage to care: an evaluation of a pilot program in South Africa. J Acquir Immune Defic Syndr **2012**; 61:e13–7.
48. Estill J, Egger M, Blaser N, et al. Cost-effectiveness of point-of-care viral load monitoring of antiretroviral therapy in resource-limited settings: mathematical modelling study. AIDS **2013**; 27:1483–92.
49. Kahn JG, Marseille EA. Viral load monitoring for antiretroviral therapy in resource-poor settings: an evolving role. AIDS **2013**; 27: 1509–11.
50. Medecins sans Frontieres. UNITAID greenlights proposal to implement POC CD4 tests and routine viral monitoring. May 10, **2012**. Available at: http://www.msf-me.org/en/news/news-media/news-press-releases/unitaid-greenlights-proposal-to-implement-poc-cd4-tests-and-routine-viral-monitoring-1.html. Accessed 7 November 2013.