Journal of Antimicrobial Chemotherapy (2009) **64**, 1126–1129
doi:10.1093/jac/dkp353
Advance Access publication 23 September 2009

Dried blood spots can expand access to virological monitoring of HIV treatment in resource-limited settings

Asgeir Johannessen[1]*, Marius Trøseid[2] and Alexandra Calmy[3,4]

[1]Ulleval Department of Infectious Diseases, Oslo University Hospital, Oslo, Norway; [2]Ulleval Medical Department, Oslo University Hospital, Oslo, Norway; [3]HIV Unit, Geneva University Hospital, Geneva, Switzerland; [4]Campaign for Access to Essential Medicines, Médecins sans Frontières, Geneva, Switzerland

The global scale-up of antiretroviral treatment in past years has, unfortunately, not been accompanied by adequate strengthening of laboratory capacity. Monitoring of treatment with HIV viral load and resistance testing, as recommended in industrialized countries, is rarely available in resource-limited settings due to high costs and stringent requirements for storage and transport of plasma. Consequently, treatment failure usually passes unnoticed until severe symptoms occur, when resistance mutations have accumulated and second-line drug options are restricted. Dried blood spots (DBS) are easy to collect and store, and can be a convenient alternative to plasma. Recently, a number of studies have demonstrated the feasibility and reliability of using DBS to monitor viral load and genotypic resistance. Moreover, several African countries have already started to use DBS for paediatric HIV screening. In the absence of point-of-care assays, the WHO should encourage virological monitoring on DBS in antiretroviral treatment programmes in resource-limited settings.

Keywords: HIV infections, antiretroviral therapy, drug resistance

The challenge: treating HIV/AIDS where there is limited laboratory capacity

Immense efforts and resources have been put into the global scale-up of antiretroviral treatment (ART) in resource-limited settings in past years. By the end of 2007, 3 million people were receiving ART in low- and middle-income countries, which is a >10-fold increase since 2002.[1] Price reductions on antiretroviral drugs from more than US$10000 to less than US$100 per person per year have been instrumental in the campaign for universal access to ART.[2] Unfortunately, the rollout of treatment programmes has not been accompanied by a similar strengthening of laboratory capacity (Figure 1). While prices for antiretroviral drugs have decreased dramatically due to generic competition, the costs of laboratory equipment needed to monitor treatment have remained high.

The honeymoon for global scale-up is over. Early treatment success has been reported from ART programmes in many low-income countries, but to uphold the success will demand a sustained effort from donor agencies as well as local ministries of health. Until now the main focus has been on quantity, such as the '3 by 5' initiative from the World Health Organization (WHO), which aimed for 3 million people on ART by 2005, and more recently the goal of universal access by 2010. However, as more and more patients are receiving ART worldwide, the focus must shift from rapid scale-up to quality-assured scale-up and ensuring long-term success of therapy.

In high-income countries, monitoring of ART with viral load and genotypic resistance testing are considered mandatory elements of patient care, in order to determine when treatment fails and tailor new regimens in failing patients.[3] These tests, however, are expensive and technically complex, and remain unavailable to the vast majority of HIV-infected individuals globally. Thus, clinicians in low-income countries are often left virtually blindfolded in the assessment of treatment failure, the need for second-line therapy and HIV status in infants.

Dried blood spots: a long-standing experience

In the absence of low-tech point-of-care assays for virological monitoring, an alternative strategy could be the shipment of specimens from peripheral clinics to central laboratories. However, stringent requirements for storage and transport of plasma are barriers in settings with a limited infrastructure. Dried blood spots (DBS) can be an alternative to plasma samples, and have been used for >40 years to screen for metabolic disorders in neonates.[4] DBS specimens are collected by spotting whole blood onto filter paper (Figure 2), either from

*Corresponding author. Tel: +47-97983264; Fax: +47-22119181; E-mail: asgeir.johannessen@medisin.uio.no

Figure 1. HIV testing in rural Tanzania with basic laboratory facilities.

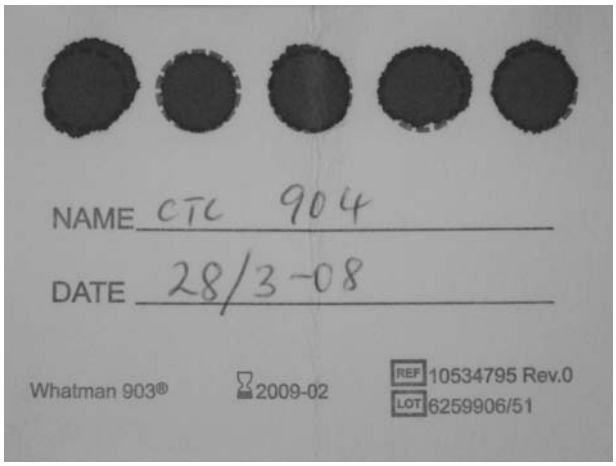

Figure 2. DBS on a Whatman 903 filter paper.

venous blood or directly from a finger prick, making this method particularly suitable in rural settings. Furthermore, DBS packed in zip-lock plastic bags with desiccant can be stored and shipped at ambient temperature, thus avoiding the need for cold chain and speedy transport to the laboratory.[5] The consumable costs for DBS are less than US$1 per test, and transport costs are markedly reduced compared with plasma, although the actual assay costs remain unchanged, and the extraction of nucleic acids from DBS involves some extra hands-on time at the central laboratory.[6,7]

Viral load quantification using DBS

Viral load measurements are mainly used to monitor the efficacy of antiretroviral therapy. When viral loads are unavailable, the WHO recommends that treatment failure should be assessed by clinical signs or CD4 cell counts.[8] However, clinical symptoms and CD4 decline have poor sensitivity and specificity in detecting virological failure.[9] Hence, patients with adequate virological suppression risk undergoing a premature switch to costly and complex second-line therapy. Furthermore, patients with actual treatment failure risk not being detected until they develop severe clinical symptoms, and prolonged exposure to a failing ART regimen leads to accumulation of resistance mutations, thus jeopardizing future treatment options.

Standard viral load assays are based on nucleic acid amplification of HIV-1 RNA. These assays are complex, expensive and require electricity, air conditioning and clean water, which usually limit their use to larger hospitals in capital cities or research projects. Newer assays based on real-time PCR or nucleic acid sequence-based amplification (NASBA) technology, although less expensive, have the same limitations. An alternative enzyme-linked immunosorbent assay (ELISA)-based assay exists, which measures reverse transcriptase activity (ExaVir Load; Cavidi AB, Uppsala, Sweden). However, this assay is time-consuming, involves several steps and the cost is still high. A dipstick PCR assay is under development by a research group at Cambridge University,[10] but this assay is not yet commercially available.

Several studies from North America and Europe have shown that DBS can be used to reliably measure viral load.[5,11–13] More recently, our own work in rural Tanzania has demonstrated the feasibility and reliability of using DBS for viral load monitoring under basic field conditions.[14] Indeed, we found that DBS, in combination with the NucliSENS EasyQ HIV-1 assay (BioMérieux, Inc., Madrid, Spain), had a sensitivity of 91% and a specificity of 97% in detecting major virological failure, defined as a plasma viral load of $\geq$5000 copies/mL. DBS have also been evaluated for non-B subtypes showing consistently satisfactory results.[14–16]

Drug resistance testing using DBS

Genotypic resistance testing is mainly used in patients who experience treatment failure, in order to tailor a new fully active regimen.[3] With an increasing number of patients receiving ART for years in resource-limited settings, the need for resistance testing, at a public health level and at an individual level for selected cases, can no longer be neglected. Switching blindly to a new regimen will become virtually impossible when patients need third- and fourth-line ART. Genotypic resistance testing is complex and expensive, and requires advanced laboratories with PCR capacity, limiting its use to major cities. Ideally, a low-tech point-of-care test should be developed, and an acceptable alternative could be a simplified assay only for selected signature mutations.

Recently, several studies have evaluated the use of DBS for drug resistance testing. In a study on HIV subtype B-infected individuals from Spain, all 46 DBS specimens from patients with a viral load of $\geq$500 copies/mL were successfully amplified using the commercial ViroSeq assay (Abbott Molecular, Des Plains, IL, USA).[17] Other studies have found amplification rates of 90.1%–92.5% in DBS specimens from Mexico and Cameroon using in-house PCR assays.[18,19] A study using the commercial Trugene HIV-1 Genotyping Kit (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) reported successful amplification in 78.8% of DBS specimens.[20] All these studies found a high concordance between resistance mutations in plasma and DBS, and an overall nucleotide similarity ranging from 98.1% to 99.95%. Our own experiences with the use of DBS in rural Tanzania support these findings; >90% of DBS specimens from patients with virological failure were

successfully amplified, and there was good agreement with mutations found in plasma.[21]

The WHO, in collaboration with a network of international experts (HIVResNet), recently published a laboratory strategy for surveillance of HIV drug resistance, where the use of DBS was recommended in resource-limited settings.[22] This method was successfully employed in surveys of transmitted resistance in Tanzania and Malawi.[23,24] However, until now, the WHO has only focused on the role of DBS in public health surveillance, and not in the clinical management of individual patients.

DBS as a tool for HIV screening in neonates

HIV-infected infants represent a particular concern with regard to diagnosis, treatment and monitoring. A major obstacle to paediatric ART is the difficulty of diagnosing HIV in infants. The persistence of maternal anti-HIV antibodies until 12–18 months of age precludes the use of rapid antibody tests, and the cost and complexity of PCR assays limit their use in resource-limited settings.[25]

Recently, several African countries have started to use DBS in order to expand the accessibility of HIV testing for infants exposed to vertical HIV transmission.[26] Detection of HIV-1 DNA in DBS is a highly sensitive and specific test of HIV infection in infants,[27] and early diagnosis and treatment is key to reducing mortality in this age group.[28] Hence, the use of DBS is already familiar to healthcare providers and laboratory technicians in several low-income countries, and this can serve as a model for further expansion of DBS monitoring strategies in such settings. Nonetheless, most HIV-exposed infants worldwide still lack access to a reliable HIV screening test, and without treatment more than one-third of HIV-infected children will not survive past their first birthday.[25]

Limitations of DBS in HIV monitoring

The use of DBS is not without caveats. First, the small amount of blood in a dried spot gives a reduced sensitivity in detecting HIV-1 RNA when the viral burden is low (<1000–4000 copies/mL).[5,13,14] On the other hand, in the updated 2006 guidelines, the WHO recommends conservation of first-line ART in resource-limited settings as long as viral load does not exceed 10 000 copies/mL.[8] We believe, therefore, that in the absence of evidence to support regimen switching at <1000 copies/mL, DBS are sufficiently sensitive to decide who needs second-line ART in such settings.

Second, since DBS consist of whole blood instead of plasma, cell-associated proviral DNA can contribute to the end product. This can explain why some studies have found false-positive viral load results in DBS.[29,30] However, this problem can be overcome by using a viral load assay based on NASBA technology, like the NucliSENS EasyQ HIV-1 assay (BioMérieux, Inc.), which is an isothermal transcription-based amplification system designed specifically for RNA detection.[31,32] With regard to resistance testing, it has been shown that proviral DNA may affect results by contributing historically archived sequences.[33,34] Nevertheless, most studies have found good agreement between genotypes generated from plasma and DBS.[17–21]

Third, adequate storage conditions for DBS have yet to be established. For viral load quantification, some studies suggest a significant loss of HIV-1 RNA after 1–3 months storage at room temperature.[11,15] In contrast, a multicenter study from North America found stable HIV-1 RNA levels in DBS stored at room temperature for at least 1 year.[5] With regard to resistance testing, recent studies showed that genotyping was highly efficient in DBS stored at 4°C for 1 year,[35] although storage at extreme conditions (37°C and 100% humidity) resulted in a rapid decline in the efficiency of genotyping after just 2 weeks.[36] For clinical monitoring of patients on ART, monthly shipment of DBS to a reference laboratory would probably be appropriate, both to avoid degradation of HIV-1 RNA, and to assure a timely switch to second-line ART in case of treatment failure.

Steps to action

There is an urgent need for virological monitoring tools developed specifically to operate under basic field conditions in resource-limited settings. Ideally, an affordable, simple and robust point-of-care assay should be developed. Donor agencies, decision-makers and the laboratory industry have a responsibility to invest in the development of laboratory equipment for low-income countries, in order to ensure the long-term success of ART worldwide.

Unfortunately, field-adapted tools for virological monitoring are not yet available. In the meantime, a monitoring strategy based on DBS is the only viable option and should be included in the next revision of the WHO guidelines, in order to meet the growing need for viral load, genotypic resistance and infant diagnosis testing in resource-limited settings. Otherwise the tremendous gains in HIV treatment in past years might be lost in the coming decade.

Transparency declarations

We declare that we have no conflicts of interest.

References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). *Report on the Global AIDS Epidemic 2008*. Geneva: UNAIDS, 2008.

2. Medecins Sans Frontieres (MSF). *Untangling the Web of Antiretroviral Price Reductions, 11th Edition, July 2008*. Geneva: MSF, 2008.

3. Hammer SM, Eron JJ Jr, Reiss P *et al*. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008; **300**: 555–70.

4. Mei JV, Alexander JR, Adam BW *et al*. Use of filter paper for the collection and analysis of human whole blood specimens. *J Nutr* 2001; **131**: 1631S–6S.

5. Brambilla D, Jennings C, Aldrovandi G *et al*. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. *J Clin Microbiol* 2003; **41**: 1888–93.

6. Buckton AJ, Prabhu DP, Cane PA *et al*. No evidence for cross-contamination of dried blood spots excised using an office hole-punch

for HIV-1 drug resistance genotyping. *J Antimicrob Chemother* 2009; **63**: 615–6.

7. Patton JC, Akkers E, Coovadia AH *et al*. Evaluation of dried whole blood spots obtained by heel or finger stick as an alternative to venous blood for diagnosis of human immunodeficiency virus type 1 infection in vertically exposed infants in the routine diagnostic laboratory. *Clin Vaccine Immunol* 2007; **14**: 201–3.

8. World Health Organization (WHO). *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach, 2006 Revision*. Geneva: WHO, 2006.

9. Mee P, Fielding KL, Charalambous S *et al*. Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa. *AIDS* 2008; **22**: 1971–7.

10. Dineva MA, Candotti D, Fletcher-Brown F *et al*. Simultaneous visual detection of multiple viral amplicons by dipstick assay. *J Clin Microbiol* 2005; **43**: 4015–21.

11. Fiscus SA, Brambilla D, Grosso L *et al*. Quantitation of human immunodeficiency virus type 1 RNA in plasma by using blood dried on filter paper. *J Clin Microbiol* 1998; **36**: 258–60.

12. Marconi A, Balestrieri M, Comastri G *et al*. Evaluation of the Abbott Real-Time HIV-1 quantitative assay with dried blood spot specimens. *Clin Microbiol Infect* 2009; **15**: 93–7.

13. Garrido C, Zahonero N, Corral A *et al*. Correlation between human immunodeficiency virus type 1 (HIV-1) RNA measurements obtained with dried blood spots and those obtained with plasma by use of Nuclisens EasyQ HIV-1 and Abbott RealTime HIV load tests. *J Clin Microbiol* 2009; **47**: 1031–6.

14. Johannessen A, Garrido C, Zahonero N *et al*. Dried blood spots perform well in viral load monitoring of patients who receive antiretroviral treatment in rural Tanzania. *Clin Infect Dis* 2009; **49**: 976–81.

15. Leelawiwat W, Young NL, Chaowanachan T *et al*. Dried blood spots for the diagnosis and quantitation of HIV-1: stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV-1 infection in Thailand. *J Virol Methods* 2009; **155**: 109–17.

16. Garrido C, Zahonero N, Fernandes D *et al*. Subtype variability, virological response and drug resistance assessed on dried blood spots collected from HIV patients on antiretroviral therapy in Angola. *J Antimicrob Chemother* 2008; **61**: 694–8.

17. Masciotra S, Garrido C, Youngpairoj AS *et al*. High concordance between HIV-1 drug resistance genotypes generated from plasma and dried blood spots in antiretroviral-experienced patients. *AIDS* 2007; **21**: 2503–11.

18. McNulty A, Jennings C, Bennett D *et al*. Evaluation of dried blood spots for human immunodeficiency virus type 1 drug resistance testing. *J Clin Microbiol* 2007; **45**: 517–21.

19. Bertagnolio S, Soto-Ramirez L, Pilon R *et al*. HIV-1 drug resistance surveillance using dried whole blood spots. *Antivir Ther* 2007; **12**: 107–13.

20. Hallack R, Doherty LE, Wethers JA *et al*. Evaluation of dried blood spot specimens for HIV-1 drug-resistance testing using the Trugene HIV-1 genotyping assay. *J Clin Virol* 2008; **41**: 283–7.

21. Johannessen A, Holberg-Petersen M, Lövgården G *et al*. Monitoring of HIV-1 drug resistance on dried blood spots is feasible and reliable in patients on antiretroviral therapy in rural Tanzania. In: *Abstracts of the Fifth International AIDS Society Conference on HIV Pathogenesis, Prevention and Treatment*, *Cape Town, South Africa*, *2009*. Abstract WEPEB210. International AIDS Society, Geneva, Switzerland.

22. Bennett DE, Myatt M, Bertagnolio S *et al*. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008; **13** Suppl 2: 25–36.

23. Somi GR, Kibuka T, Diallo K *et al*. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. *Antivir Ther* 2008; **13** Suppl 2: 77–82.

24. Kamoto K, Berle-Grasse J. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. *Antivir Ther* 2008; **13** Suppl 2: 83–7.

25. Sutcliffe CG, van Dijk JH, Bolton C *et al*. Effectiveness of antiretroviral therapy among HIV-infected children in sub-Saharan Africa. *Lancet Infect Dis* 2008; **8**: 477–89.

26. Stevens W, Sherman G, Downing R *et al*. Role of the laboratory in ensuring global access to ARV treatment for HIV-infected children: consensus statement on the performance of laboratory assays for early infant diagnosis. *Open AIDS J* 2008; **2**: 17–25.

27. Sherman GG, Stevens G, Jones SA *et al*. Dried blood spots improve access to HIV diagnosis and care for infants in low-resource settings. *J Acquir Immune Defic Syndr* 2005; **38**: 615–7.

28. Violari A, Cotton MF, Gibb DM *et al*. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med* 2008; **359**: 2233–44.

29. Waters L, Kambugu A, Tibenderana H *et al*. Evaluation of filter paper transfer of whole-blood and plasma samples for quantifying HIV RNA in subjects on antiretroviral therapy in Uganda. *J Acquir Immune Defic Syndr* 2007; **46**: 590–3.

30. Monleau M, Montavon C, Laurent C *et al*. 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**: 1107–18.

31. Bruisten S, van Gemen B, Koppelman M *et al*. Detection of HIV-1 distribution in different blood fractions by two nucleic acid amplification assays. *AIDS Res Hum Retroviruses* 1993; **9**: 259–65.

32. van Gemen B, Wiel P, van Beuningen R *et al*. The one-tube quantitative HIV-1 RNA NASBA: precision, accuracy, and application. *PCR Methods Appl* 1995; **4**: S177–84.

33. Buckton AJ, Bissett SL, Myers RE *et al*. Development and optimization of an internally controlled dried blood spot assay for surveillance of human immunodeficiency virus type-1 drug resistance. *J Antimicrob Chemother* 2008; **62**: 1191–8.

34. Steegen K, Luchters S, Demecheleer E *et al*. Feasibility of detecting human immunodeficiency virus type 1 drug resistance in DNA extracted from whole blood or dried blood spots. *J Clin Microbiol* 2007; **45**: 3342–51.

35. Youngpairoj AS, Masciotra S, Garrido C *et al*. HIV-1 drug resistance genotyping from dried blood spots stored for 1 year at 4°C. *J Antimicrob Chemother* 2008; **61**: 1217–20.

36. Garcia-Lerma JG, McNulty A, Jennings C *et al*. Rapid decline in the efficiency of HIV drug resistance genotyping from dried blood spots (DBS) and dried plasma spots (DPS) stored at 37°C and high humidity. *J Antimicrob Chemother* 2009; **64**: 33–6.