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Commentary

Dried blood spot testing: filling the gap between antiretroviral treatment & monitoring in India

Antiretroviral coverage in low-middle income countries has significantly been expanded in recent years, substantiated in the ambitious United Nations commitment to complete universal access to HIV care by 2015[1]. While decreased cost of drugs, availability of generic drugs, expanded foreign assistance and improved local health policies are expected to facilitate this goal, a key paradigm of the antiretroviral treatment (ART) success achieved in western countries is the integration between therapy and virological monitoring[2]. Specifically, quantification of HIV RNA in plasma (viraemia) has been long recognized as the key surrogate parameter of the course of HIV infection. The definition of virological failure or success of antiretroviral treatment is indeed based on plasma HIV RNA itself. Viraemia and CD4 cell counts are the reference parameters, together with genotypic drug resistance testing[3], to obtain the maximum benefit from access to therapy.

Measurement of HIV RNA requires the availability of a robust molecular system certified for *in vitro* diagnostic use and the possibility to store and send plasma samples in a well-controlled cold chain system. One alternative to processing and shipment of plasma is to spot and dry blood on filter paper and have HIV RNA retrieved and measured later from this dried blood spot (DBS) matrix. The advantage of the system is that nucleic acids are relatively stable in DBS and thus the requirement for a cold chain during storage and transportation is not necessary. This in turn facilitates access to HIV RNA testing in resource-limited settings, particularly rural areas where local laboratory facilities are scarce or absent and the reference laboratory is difficult to reach. Measurement of HIV RNA in DBS was first described in 1997[4] and has been increasingly documented as a reliable methodology in recent years through simulated experiments in western countries or field testing in low-income countries, particularly in Africa[5,6]. Importantly, the latest studies have all adapted certified state-of-the-art HIV RNA assays[7-10] rather than developing in house methods. The latter, although attractive in terms of cost reduction, may add the need for an extra optimization effort and potentially lead to lower accuracy with respect to certified commercial systems.

The paper by Neogi *et al*[11] in this issue follows this line showing feasibility and accuracy of DBS HIV RNA quantification via the Abbott m2000rt assay in the Indian settings. The results are very similar to those reported by the same method few months ago by another Indian group[12]. Both studies used manual extraction, instead of the integrated m2000sp automated platform usually coupled with this system, yet the correlation with the reference liquid plasma HIV RNA values was high. Neogi *et al*[11] showed that the difference in viraemia between the paired samples was within 0.5 log in 80 per cent of the cases and >1.0 log in no case, similar to what also the other Indian group reported earlier[12]. Among current HIV RNA assays, the Abbott system is the one which has been used on DBS more frequently[7,11-15].

In general, most of the studies comparing DBS vs. liquid plasma for HIV RNA quantification have so far yielded satisfactory results, supporting the use of DBS when plasma cannot be properly stored and shipped. The limitations of the DBS approach have also been reviewed[16]. First, the small volume of blood spotted (usually 50 microliters per spot) and the low efficiency of RNA extraction from multiple spots significantly limit the concentration of the RNA extract with respect to standard processing of liquid plasma. This results in lower sensitivity of DBS testing, with detection of HIV RNA in about 50 per cent of the cases where plasma HIV RNA is around 3 log. Second, correlation with

plasma HIV RNA values, and thus accuracy, decreases with lower viraemia levels. This is partly due to the same reason (small blood volume analyzed) and partly complicated by the inability to distinguish between HIV RNA and DNA which can falsely increase the test result[12]. Third, although nucleic acids in DBS are relatively stable, detailed studies[17,18] have reported variable HIV RNA degradation with prolonged storage at room temperature, particularly in high humidity conditions that can be frequently found in many resource-limited settings.

The third issue appears to be easily addressed, assuming that even remote rural regions can store DBS in a refrigerator as long as needed. In addition, DBS HIV RNA testing in clinical practice must be performed as timely as possible to be clinically useful. Thus, prolonged storage would probably not occur in this setting, limiting the impact even of storage at high temperature. On the other hand, the issues related to the low amount of blood analyzed and the unpredictable level of contaminating HIV DNA in the DBS do not have an easy technical solution. HIV DNA could be removed by DNase digestion but this requires an extra step currently not included in any certified HIV RNA quantification system (because it is not needed when working with plasma) and would considerably complicate DBS handling. In theory, the amount of plasma, and hence the assay sensitivity, could be increased by processing multiple DBS. However, collection and excision of many spots is highly impractical and, when we first adapted the Abbott assay to DBS[7], we found no increase in RNA concentration using more than four spots because of the annoyances with a large amount of paper requiring large volumes of buffer (personal data). Limited sensitivity and possible inaccuracies due to contaminating HIV DNA at low HIV RNA levels result in difficulty to detect early virological failure of antiretroviral treatment, *i. e.* low-level HIV RNA rebound. At this time, it is difficult to anticipate what, if any, can be the consequences of a potentially delayed diagnosis of virological treatment failure in the resource-limited setting. Although remaining on a failing regimen for a prolonged period carries an inherent risk of drug resistance and cross-resistance development[19], the sensitivity of DBS HIV RNA testing should be enough to provide an effective guide to treatment change when needed.

In summary, the potential drawbacks associated with the use of DBS for HIV RNA quantification appear to be largely outweighed by the benefits derived from increased access to virological monitoring of antiretroviral treatment in resource-limited settings. To warrant adequate accuracy, certified HIV RNA quantification systems should be probably preferred over in house technology, unless exhaustive optimization and comparative analyses with a reference system have been successfully completed. Also, automated RNA extraction should be preferred over manual systems which, by definition, are much more prone to human error and inconsistencies. Irrespective of the assay used, local authorities should develop the appropriate quality assurance programmes to ensure that the laboratories involved have and maintain a good performance in DBS HIV RNA testing. The use of certified systems and automated extraction procedures clearly requires a larger financial effort with respect to in house assays and manual procedures. However, the expanding use of the reference systems and the possible development of novel certified systems by other companies can reasonably result in much lower costs in a short time. Such cost savings will add to savings derived from avoidance of the cold chain transportation allowed by the DBS format. Thus, quantification of HIV RNA in DBS can be a valid and cost-effective choice in many low-middle income countries aiming at complementing expanded access to antiretroviral treatment with the required virological monitoring.

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