Evaluation of the Abbott Real-Time HIV-1 quantitative assay with dried blood spot specimens

A. Marconi1, M. Ballestri2, G. Comastri2, F. R. Pulvirenti2, W. Gennari3, S. Tagliazucchi3, M. Pecorari3, V. Borghi4, D. Marri5 and M. Zazzi6

1) Department of Molecular Biology, University of Siena, Siena, 2) Abbott Molecular Diagnostics, Rome, 3) Unit of Microbiology, Modena University Hospital, Modena, 4) Division of Infectious Diseases, Modena University Hospital, Modena and 5) Division of Infectious Diseases, Siena University Hospital, Siena, Italy

Abstract

The Abbott Real-Time HIV-1 assay was evaluated for its performance in quantification of human immunodeficiency virus type I (HIV-1) RNA in dried blood spot (DBS) samples. In total, 169 blood samples with detectable plasma HIV-1 RNA were used to extract RNA from paired DBS and liquid plasma samples, using the automated Abbott m Sample Preparation System (m2000sp). HIV-1 RNA was then quantified by the m2000rt RealTime analyser. RNA samples suitable for real-time PCR were obtained from all but one (99.4%) of the DBS samples and HIV-1 RNA was detected in 163/168 (97.0%) samples. The correlation between HIV-1 RNA values measured in paired DBS and plasma samples was very high (r = 0.882), with 78.5% and 99.4% of cases differing by <0.5 and 1.0 log, respectively. Retesting of DBS replicates following 6 months of storage at 2–8°C showed no loss of HIV-1 RNA in a subset of 89 samples. The feasibility of DBS testing coupled with automated sample processing, and the use of a latest-generation FDA-approved real-time PCR-based system, represents an encouraging first step for viral load measurement in reference centres in developing countries where access to antiretroviral therapy is expanding.

Keywords: Antiretroviral therapy, dried blood spot, human immunodeficiency virus, monitoring, viral load assay

Original Submission: 11 June 2008; Revised Submission: 3 July 2008; Accepted: 3 July 2008

10.1111/j.1469-0691.2008.02116.x

Corresponding author and reprint requests: M. Zazzi, Division of Microbiology, Department of Molecular Biology, University of Siena, Viale Bracci 1, 53100 Siena, Italy
E-mail: zazzi@unisi.it

The epidemic of human immunodeficiency virus type I (HIV-1) infection in Africa and other countries with limited healthcare resources remains a major cause of morbidity and mortality [1]. The elevated cost of antiretroviral drugs has been one of the main obstacles to the control of the epidemic in these areas. Nonetheless, there is now increasing access to antiretroviral therapy through health programmes funded by the WHO and national health services, as well as through international charity foundations [2]. Systematic monitoring of viral load, as measured by plasma HIV-1 RNA amount, is the standard method for verifying adequate control of viral replication in patients under antiretroviral treatment (http://www.aidsinfo.nih.gov/Guidelines/). Indeed, viral load has been shown to be predictive of clinical progression, and to change promptly in response to treatment success or failure. Thus, expanding access to antiretroviral therapy in developing countries must be supported by the availability of methods to quantify viral load. However, monitoring of viral load in countries with limited resources poses logistical challenges. Relatively few laboratories that have the capacity to perform molecular diagnostics exist. Such laboratories are often distant from rural communities, which account for a large proportion of the population. The difficulty in ensuring the necessary conditions of transport between the site of blood sampling in local dispensaries and the monitoring laboratory may preclude routine assay of liquid plasma samples. In addition, access to a reliable source of electricity and to clean water may be limited in rural areas.

One solution that has been proposed for improving access to viral load monitoring in such countries is the use of dried blood spots [3–10]. A drop of blood can be obtained in the field from a finger prick, applied to a piece of filter paper, air-dried, and stored until measurement of HIV-1 RNA. It has been demonstrated that HIV-1 RNA in dried blood spot (DBS) samples is stable over time under differing conditions of temperature and humidity [4,6,9]. The feasibility of HIV-1 RNA testing in DBS samples that can be easily transported makes possible the establishment of reference centres where state-of-the-art molecular diagnostics can be used to quantify HIV-1 RNA in patients scattered throughout large geographical areas.

FDA-approved methods for quantification of HIV-1 RNA were introduced in 1996 [11]. Since then, molecular diagnostic methods have evolved considerably. Nowadays, real-time amplification, coupled with automated extraction, offers several advantages over conventional PCR, including a lower risk of contamination, a more rapid turnover time, less stringent requirements concerning technical skills on the part of operators, and an extended dynamic range [12,13].
Although real-time PCR has become the preferred technology for quantification of HIV-1 RNA in a clinical diagnostic setting, there have been no reports of its use with DBS samples. The objective of this study was to evaluate the feasibility of using the Abbott Real-Time HIV-1 assay to quantify HIV-1 RNA load in DBS samples, as compared with liquid plasma samples, in an Italian test population.

The study was conducted at two HIV reference centers in Italy at the University Hospitals of Modena and of Siena. Citrated blood samples were collected from the HIV-1-infected patients presenting at one of the two study centers during a pre-established enrollment period who were found to harbor at least 200 copies/mL of plasma HIV-1 RNA. The total number of patients enrolled was 169 (95 in Modena and 74 in Siena). The study protocol was approved by the local ethics committee, and all patients gave informed consent to participate in the study. One milliliter of plasma was assayed for detection of HIV-1 RNA, as usual after a single freeze–thaw cycle. Two or four further 50-µL aliquots of whole blood were applied to Whatman 903 filter-paper cards, which were stored for a variable duration (median, 26 days; range, 1–170 days) at room temperature before assay. A subset of 89 samples, for which multiple DBS samples were obtained at collection, was used to investigate the stability of HIV-1 RNA in DBS samples for 6 months at 2–8°C.

For the DBS samples, a half-inch disk entirely covered with the sample was cut from each spot. Two disks (one each from the two spots prepared from the same patient) were incubated in 2 mL of mLysis buffer provided with the Abbott m Sample Preparation System (m2000sp) in 50-mL sealed conical tubes. The tubes were incubated at room temperature for 2 h, with intermittent mixing, and the entire contents were then transferred to an m2000sp reaction vessel. The lysate was processed according to the standard HIV-1 RNA 1.0-mL extraction protocol as described elsewhere [12]. The paired liquid plasma samples were tested, placing them directly into the m2000sp apparatus, using the same 1.0-mL routine protocol. The m2000sp then mixed the RNA extracts obtained with the amplification reagents and dispensed the resulting reaction mixtures onto the Abbott 96-Well Optical Reaction Plate. An armoured RNA sequence unrelated to the HIV-1 target sequence was introduced into each specimen at the beginning of sample preparation as an internal control. A negative control (HIV-1-negative human plasma) and a low and a high positive control (non-infectious armoured RNA with inserted HIV-1 target sequences diluted in HIV-1-negative human plasma) were included in each test run for assay validation.

The 96-well m2000sp reaction plate was transferred to the m2000rt RealTime analyser for RT-PCR amplification of HIV-1 RNA. The amount of HIV-1 target sequence that is present at each amplification cycle is quantified by measuring the fluorescence of the HIV-1 probe that binds to the target during the extension/anneal step. The 1.0-mL HIV-1 RNA protocol was used in accordance with the manufacturer’s instructions. Fluorescence counts were converted directly into viral load measures by the m2000rt analyser. The results obtained with the DBS samples were corrected in order to take into account the different input volumes used. A correction factor based on the difference between liquid plasma and dry spot measurements of the high positive kit control, tested in duplicate in each run, was calculated. This factor was highly reproducible; its mean ± SD in five runs was 1.94 ± 0.06 log copies/mL. On the basis of this evidence and additional in-house data, Abbott Molecular has made available an m2000 DS HIV-1 RNA ‘open-mode’ protocol with a software-embedded correction factor, so that no manual data correction is needed before releasing the results.

A subset of 163 of the 169 (0.6%) samples tested. HIV-1 RNA was detected in 163 of the 168 DBS samples, with 97.0% sensitivity (Table 1). The correlation between viral load values obtained from the paired 163 liquid plasma and DBS samples was high (Pearson product–moment correlation coefficient = 0.882). Linear regression analysis of the viral loads obtained with the two types of sample yielded a slope of 0.852 and an intercept of 0.616 (Fig. 1). The mean ± SD differ-

### TABLE 1. Human immunodeficiency virus type 1 RNA detection rates in paired dried blood spot (DBS) and liquid plasma samples

<table>
<thead>
<tr>
<th>Viral load in liquid plasma</th>
<th>No. of samples</th>
<th>DBS-positive</th>
<th>Detection rate, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200–1000 copies/mL</td>
<td>20</td>
<td>18</td>
<td>90.0 (68.7–98.4)</td>
</tr>
<tr>
<td>1000–10 000 copies/mL</td>
<td>57</td>
<td>54</td>
<td>94.7 (85.1–98.8)</td>
</tr>
<tr>
<td>10 000–100 000 copies/mL</td>
<td>75</td>
<td>75</td>
<td>100.0 (94.2–100.0)</td>
</tr>
<tr>
<td>&gt;100 000 copies/mL</td>
<td>16</td>
<td>16</td>
<td>100.0 (77.3–100.0)</td>
</tr>
<tr>
<td>All samples</td>
<td>168</td>
<td>163</td>
<td>97.0 (93.0–98.9)</td>
</tr>
</tbody>
</table>
The difference between the viral load measured in the DBS samples and that determined in liquid plasma was 0.01 ± 0.39 log copies/mL. Overall, viral load values in the two sample types differed by less than half a log unit in 78.5% (128/163) of the samples and by less than one log unit in 99.4% (162/163) of the samples. However, the differences between DBS and plasma HIV-1 RNA were higher in the 200–1000 copies/mL range, as compared with the >1000 copies/mL group (-mean ± SD = 0.50 ± 0.35 vs. −0.05 ± 0.35; p <0.001) (Fig. 2).

To assess the stability of HIV-1 RNA in DBS samples, a subset of 89 samples with multiple spots was retested after 6 months of storage at 2–8°C, following the first DBS analysis. The correlation coefficient derived from the two consecutive measurements was 0.955, with a slope of 0.963 and an intercept of 0.150 (Fig. 3). The mean (±SD) difference between the first and second measurement was 0.01 (±0.22) log. All of the paired values were within 1 log difference, and only five (5.6%) samples differed by >0.5 log.

The feasibility study reported here demonstrates, for the first time, that HIV-1 RNA can be quantified in DBS samples using a state-of-the-art FDA-approved real-time PCR assay, the Abbott Real-Time HIV-1 assay. Although the samples analysed were obtained from patients living in an area where HIV-1 subtype B is still highly prevalent, the choice of this assay from among the latest-generation systems was based on its excellent performance in assessing different HIV-1 groups and subtypes in previous studies [12,17]. Indeed, the highly diversified nature of HIV-1 in most of the developing countries [18] poses a major challenge to accurate quantification of all the distinct genetic variants and requires rigorous validation of any assay in this context.

This study revealed a very good correlation between liquid plasma and DBS measurements, an excellent RNA stability over a 6-month period, and a definitely acceptable threshold of sensitivity in light of the source material and the clinical setting in which this assay would be used. Although a formal definition of the lower limit of quantification has not been established at this stage, a 90% detection rate was achieved with samples containing 200–1000 copies of HIV-1 RNA/mL, a performance comparable to, or better than, that...
of the first-generation assays successfully used in Western countries for several years. Nevertheless, the limited amount of sample analysed by DBS testing necessarily leaves a small proportion of patients in whom detection of early treatment failure can be achieved with standard plasma testing but not with the DBS assay. Although the blood adsorption capacity of the filter paper disks does not allow the use of larger amounts of blood, the overall sample volume could be increased by using multiple spots. However, the amount of lysis buffer should also be increased, due to augmented paper adsorption. Thus, the possibility of obtaining a more concentrated RNA sample needs to be carefully investigated.

A larger difference between DBS and plasma HIV-1 RNA was noticed in the 200–1000 copies/mL range than in the >1000 copies/mL samples; this may be explained by the contribution of intracellular HIV-1 DNA and RNA which, by definition, is present in the DBS but not in the plasma counterpart. As intracellular HIV-1 DNA and RNA may be detectable at low or undetectable levels of plasma viraemia [19–21], their contribution could be more relevant to these samples than to samples containing higher levels of extracellular HIV-1 RNA. Formal quantitative analysis of cellular HIV-1 DNA and RNA, as opposed to plasma HIV-1 RNA, in samples to be analysed in the form of DBS samples and plasma is required to evaluate this hypothesis.

This study was undertaken as a first step to assess the feasibility of DBS testing by an FDA-approved latest-generation real-time PCR-based system. Although excellent results have been shown here, several additional factors must be considered in the application of this strategy in developing countries. First, the storage conditions evaluated here may be different from those required in some specific settings. Although HIV-1 RNA has been shown to be stable in DBS samples under different temperature and storage conditions, in this and previous studies [4,6,9], it will be important to replicate the current results in a tropical setting, using routine operational and logistic procedures. Second, an automated RNA extraction system was used, according to current recommendations, to minimize human intervention and operator-dependent variability. Although the same strategy may be conceivable for reference centres in developing countries, with the advantage of a decreased need for major investment in skilled personnel, local policies may prevent the equipment of laboratories with automated sample-processing instruments. Thus, the reproducibility of DBS analysis should be tested with commonly used and effective manual RNA extraction protocols [22]. In this context, Abbott Molecular is providing a certified manual extraction method as an alternative to automated processing (Abbott Real-Time HIV-1 Package Insert; Abbott Molecular Inc., Des Plaines, IL, USA, May 2007). Notwithstanding the need for further analysis, quantification of HIV-1 RNA in DBS samples by the Abbott Real-Time HIV-1 assay appears to be a promising strategy to support the expanding access to antiretroviral therapy in developing countries.

Acknowledgements

Reagents and protocols were kindly provided by Abbott Molecular. This work was presented in part at the First Interest Workshop—International Workshop on HIV Treatment, Pathogenesis and Prevention Research in Resource-poor Settings, Kampala, Uganda, 30 May to 2 June 2007.

Transparency Declaration

This work was supported, in part, by grant 30G.58 (VI Programma Nazionale di Ricerca AIDS 2006, Italian Ministry of Health) and by the Academic Research Program 2005 of the University of Siena, Italy. G. Comastri and F. R. Pulvirenti are employees of Abbott Molecular, Italy. M. Zazzi has been a consultant for Abbott Molecular, Italy.

References

An outbreak of norovirus infection in an Italian residential-care facility for the elderly

M. C. Medici¹, A. Morelli², M. C. Arcangeletti¹, A. Calderaro¹, F. De Conto¹, M. Martinelli¹, L. A. Abelli¹, G. Dettori³ and C. Chezzi¹

¹) Section of Microbiology, Department of Pathology and Laboratory Medicine, University Medical School of Parma, Viale Antonio Gramsci, 14, 43100 Parma, Italy
²) Public Hygiene Service, Department of Public Health, Local Health Unit, Langhirano, Parma, Italy

Abstract

On December 2006, an outbreak of gastroenteritis occurred at a residential-care facility for the elderly in northern Italy. Thirty-five of 61 individuals interviewed (attack rate, 57.4%) fell ill. In 94.3% of cases, the onset of illness was within 48 h of a Christmas party at the facility. Norovirus (NoV) was detected by RT-PCR in 24 of 31 individuals examined, including three asymptomatic food-handlers, in whom there was evidence of long-lasting excretion of viral particles. The identification of a sequence referring to the ‘2006a GII.4 NoV variant’ in all examined strains supported the hypothesis of a common point source. This retrospective cohort study is the first report on an outbreak of NoV gastroenteritis in an Italian residential-care facility for the elderly.

Keywords: Asymptomatic infection, norovirus outbreak, residential-care facility, sequence analysis, virus shedding

Original Submission: 22 May 2008; Revised Submission: 23 June 2008; Accepted: 5 July 2008

Editor: J.-C. Desenclos

Clin Microbiol Infect 2009; 15: 97–100
10.1111/j.1469-0691.2008.02117.x

Corresponding author and reprint requests: M. C. Medici, Section of Microbiology, Department of Pathology and Laboratory Medicine, University Medical School of Parma, Viale Antonio Gramsci, 14, 43100 Parma, Italy
E-mail: mariacristina.medici@unipr.it

Outbreaks of gastroenteritis due to norovirus (NoV) often occur during the winter months, and take place in closed and semi-closed settings. This retrospective cohort study describes an outbreak of gastroenteritis caused by NoV at a residential-care facility for the elderly in northern Italy. On 22 December 2006, the manager of the facility reported the occurrence of several cases of acute gastroenteritis among residents. All individuals had attended a Christmas party on 20 December at the facility. A case was defined as an illness in any resident, staff member or guest who took part in the event and experienced diarrhoea (three or more loose stools within 24 h) and/or vomiting. The food served on that occasion by individual serving (different food for residents and for staff members and guests) was suspected to be