Journal of Virological Methods 193 (2013) 439–445

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

# Evaluation of dried blood spot diagnosis using HIV1-DNA and HIV1-RNA Biocentric assays in infants in Abidjan, Côte d'Ivoire. The Pedi-Test DBS ANRS 12183 Study☆

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*Article history:*
Received 8 October 2012
Received in revised form 13 May 2013
Accepted 3 July 2013
Available online 16 July 2013

*Keywords:*
HIV
Infant diagnosis
Africa
Diagnosis performances

A B S T R A C T

This study evaluates HIV infant diagnosis on DBS using Biocentric HIV1-DNA and HIV1-RNA assays, in field conditions in Côte d'Ivoire.

Paediatric screening was offered to children ≤3 years in clinical sites in Côte d'Ivoire in 2008. For each HIV-infected child, two non-infected children were included and blood samples were collected. HIV-DNA results obtained on EDTA blood samples with Biocentric assay were the reference for HIV infant diagnosis. Plasma and DBS viral loads were measured using HIV-RNA Biocentric assay. DBS samples were also tested for HIV-DNA detection using both Biocentric and Amplicor Roche assays. Sensitivity, specificity and concordance between tests were calculated.

Overall samples from 138 HIV-exposed children, 46 infected, 92 non-infected were included. All tests were 100% sensitive and specific including 100% concordance with the two HIV-DNA assays. The median level of HIV-DNA on EDTA samples was 3.15 $\log_{10}$ copies/$10^6$ PBMCs; the median level of HIV RNA in plasma and DBS were respectively 5.82 and 5.17 $\log_{10}$ copies/ml (Pearson's correlation $R^2 = 0.92$, $p < 0.0001$). The threshold for detectable HIV-RNA on DBS was 3.3 $\log_{10}$.

Although there are differences between viral load measured on DBS and plasma, the two Biocentric assays present very good performances for HIV infant diagnosis on DBS while cheap and feasible.

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## 1. Introduction

According to the 2011 UNAIDS report 390,000 children were newly infected with HIV in the world, 90% of whom in sub-Saharan Africa, despite the scaling up of Prevention of Mother-To-Child-Transmission (PMTCT) programmes (UNAIDS, 2011). In the absence of antiretroviral therapy (ART), HIV-related child mortality in Africa is early and dramatically high, reaching 35% at 12 months and 52% by 2 years (Newell et al., 2004a,b). Early diagnosis of HIV-1 infection in infants born to seropositive mothers is essential for preventing early HIV-related mortality by allowing early initiation of appropriate ART (Faye et al., 2004; Violari et al., 2008). In 2010, the World Health Organisation (WHO) recommended systematic HIV-1 diagnosis for all infants aged 4 to 6 weeks, using tests with a sensitivity of at least 95%, followed by immediate ART initiation (WHO, 2011a,b). Serological testing before the age of 18 months is not reliable because of the presence of maternal antibodies in the baby's blood; therefore early infant diagnosis requires specific and sophisticated virological assays involving polymerase chain reaction (PCR) techniques, which can confirm an HIV diagnosis from 4 to 6 weeks of life (WHO, 2011a,b). It has been estimated that in the

☆ Results of this study were presented in part at the 16th International Conference on AIDS & STD in Africa (ICASA); 2011; Addis Ababa, Ethiopia, 4–8th December 2011, Poster Abstract TUPE004 and the 4th International Workshop on HIV Pediatrics; Washington, DC, USA, 20–21 July 2012. Poster Abstract number: P-99.

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http://dx.doi.org/10.1016/j.jviromet.2013.07.011

absence of antiretroviral prophylaxis, 95% of HIV-1 infections can be detected by PCR at 2–4 weeks of age, with the exception of infants infected via breastfeeding (Dunn et al., 1995, 2000; Tournoud and Ecohard, 2006). Detection of HIV-1 DNA in cells and HIV-1 RNA in plasma has been used to diagnose HIV-1 infection in neonates (Delamare et al., 1997; Young et al., 2000).

Early access to ART is therefore conditioned by access to early infant diagnosis, which has been described by previous studies as very poor in Côte d'Ivoire (The KIDS-ART-LINC Collaboration, 2008; Anaky et al., 2010; Stringer et al., 2010). Improving early infant diagnosis remains a priority in the management of paediatric HIV in resource-limited settings, where the epidemic continues to grow (Anaky et al., 2010). The most used test for early infant diagnosis is the Amplicor HIV-1 DNA test 1.5 (Roche Diagnostics, Indianapolis, Indiana, USA). This assay is however very time – consuming and other more simple diagnostic tests based on real time PCR technology have been developed (Rouet et al., 2005, 2007; Ou et al., 2007; Johannessen et al., 2009; Lofgren et al., 2009; Kébé et al., 2011). Moreover, venous blood sampling from infants is invasive, often difficult and involves conditioning in the laboratory before any analyses can be carried out; few laboratories in Côte d'Ivoire are equipped accordingly. For these reasons, capillary blood collected on dried blood spots (DBS) is an easier option that has been preferred in Côte d'Ivoire to support the scaling-up of the HIV Care National Programme. The French Agency for Research on AIDS and viral hepatitis (ANRS) has developed generic HIV-1 DNA and HIV1-RNA tests marketed by Biocentric (Bandol, France) which are achievable in one working day (5–6 h for 84 samples versus 2–3 days for the Amplicor HIV-1 DNA test) (Ou et al., 2007). In addition to the capability for early infant diagnosis, these assays can allow the quantification of plasma HIV-RNA (viral load) and cellular HIV-DNA (cell viral load) making profitable the same equipment and reagents. These assays have already been developed and validated on EDTA blood samples in Côte d'Ivoire, a region with a high HIV-1 genetic diversity (Rouet et al., 2007; Toni et al., 2007). However no studies have validated them on DBS samples taken routinely in children for early infant diagnosis in Côte d'Ivoire.

In 2008, the ANRS 12165 Pedi-Test project was launched in Côte d'Ivoire, evaluating the acceptability of routine HIV screening during vaccination or postnatal consultations for children and their families, considering a child-centred strategy. Subsequently, the ANRS 12183 Pedi-Test-DBS study was set up as part of this project, with the objective of evaluating the feasibility and diagnostic performances of DBS testing for early infant diagnosis compared to EDTA blood samples using the two Biocentric tests in resource-limited settings, where molecular biology facilities are limited.

## 2. Materials and methods

### 2.1. Study patients

Inclusion criteria were HIV-1 exposed-children, aged 4 weeks to 36 months between March and October 2008. Samples were obtained from different paediatric cohorts in Côte d'Ivoire: the Pedi-Test project (ANRS 12165), the TEmAA project (ANRS 12109), and the PMTCT Plus Programme, CePReF and CIRBA paediatrics cohorts. Seven clinical sites were involved, six in Abidjan (districts of Abobo, Koumassi, Treichville and Yopougon) and one in Bonoua (a suburb of Abidjan).

### 2.2. Study design

Voluntary HIV serological testing was offered to mothers and their child during vaccination and monthly postnatal visits. In case of orphans, HIV serological testing was offered to the child only. All HIV-exposed children had an EDTA blood sample and a capillary DBS performed to diagnose HIV infection. For this, the reference method of HIV-infection diagnosis was the detection of HIV-DNA quantified on cell pellets from whole EDTA blood samples by the Generic HIV-DNA Cell kit (Biocentric, Bandol, France). This method allowed to identify both HIV-1 – infected and non-infected children. For each HIV-infected child, two non-infected children were selected at random and included in the study. First, the diagnosis performances of the four tests were assessed and compared: detection of cell HIV-DNA on DBS, and qualitative Amplicor HIV-1 DNA Test v1.5 (Roche Diagnostics, Indianapolis, Indiana, USA) tests compared to the reference method for early infant diagnosis. Second, HIV-RNA viral loads on EDTA plasmas and on DBS were measured using the Generic HIV Charge Virale RNA assay (Biocentric, Bandol, France). Results were compared to the reference method and agreement between both viral load measurements was assessed.

### 2.3. Biological study procedures

HIV serological testing was carried out using rapid HIV antibody tests according to the national guidelines (Public Health Ministry of Cote d'Ivoire). These guidelines recommend the use of two rapid tests, Determine HIV1/2 test [Abbott, North Chicago, IL, USA] and Genie II HIV-1/HIV-2 [Bio-Rad, Hercules, CA, USA]. These tests allowed the identification of HIV-exposed children to whom venous blood was collected on tubes and capillary blood was collected on 903-protein saver cards [Schleicher & Schuell, Whatman, Versailles, France] (Whatman, 2009). The tubes were conveyed within the same day to the laboratory in a cooled container while the DBS were sent in paper envelopes at ambient temperature within 1–15 days after blood collection. Rate of arrivals for DBS to the laboratory (number of DBS received and number of DBS in line with the national recommendations) and the quality of the received DBS (number of good, average and bad DBS) were measured. DBS that fell into the following criteria were rejected: less than three spots of blood, coagulated, contaminated, diluted, discoloured and dirty blood spot, insufficient quantity of blood, poorly labelled DBS, no patient identification or no date of collection. Blood collected on EDTA-containing tubes was plasma-decanted and centrifuged for 10 min at 2500 rpm to separate plasma from cell pellet. DBS, plasma and cell pellets were stored at −80 °C until processing. Cell pellets were chosen instead of Ficoll-Hypaque separation to save infant plasma and on the basis that HIV-DNA quantitation estimated in whole blood and expressed in $\log_{10}$ copies/$10^6$ cells was highly correlated with that obtained after Ficoll-Hypaque separation (Avettand-Fènoël et al., 2009).

### 2.4. Assays

#### 2.4.1. Generic HIV-RNA charge virale assay

Viral RNA was extracted from plasma and DBS, by the QIAamp Viral RNA mini kit [Qiagen, Cortaboeuf, France] as describe by the manufacturer's recommendations (Rouet et al., 2007). QIAamp DNA mini kit [Qiagen, Courtaboeuf, France] was used for total DNA extraction in DBS, as recommended by the manufacturer. Briefly, one spot was partially (approximately 75%) punched out to obtain 3 circles placed into a 1.5 ml tube containing 180 μl of buffer ATL (tissue lysing buffer). The tube was incubated for 10 min at 85 °C before and 20 μl of Proteinase K stock solution, and then incubated for one hour at 56 °C. Then 200 μl of buffer AL (cell and viral lysis buffer) were added. After 10 min of incubation at 70 °C, 200 μl of ethanol were added to precipitate the nucleic acids. Five centrifugations at different speeds were made, followed by the addition of 150 μl of buffer AE (elution buffer) on the QIAamp Mini spin column before the last centrifugation. This last step allowed collecting 150 μl of eluate solution of nucleic acids that can be processed in

downstream applications (RNA and DNA detection). RNA quantitation was conducted by a one-step RT-PCR real time assay with specific primers and Taqman probe located in the HIV long terminal repeat regions (LTR) performed by the Applied Biosystems open Real Time PCR system named ABI Prism 7000 SDS (Applera International, Cortaboeuf, France). The manufacturer provided quantitative standards to determine the plasmatic viral load.

The amount of plasma in each spot of DBS was determined on the basis of the paediatric population's mean haematocrit at 50% assuming that the amount of plasma in each spot differed by less than 10% when haematocrit ranged from 30 to 50% as shown by Mei et al. (2001). Furthermore, when saturated, each circle or spot of 903 protein card saver hold 75–80 μl of whole blood, 80 μl were chosen for this study; thus, for 75% of one spot used, 30 μl corresponding plasma were used versus 200 μl of plasma for plasmatic viral load (Whatman, 2009). The correcting factor was 6.67 (200/30) and DBS viral load was calculated by multiplying the number displayed by the ABI Prism by the correction factor. The limit of detection was estimated at 300 copies/ml in plasma as previously describe (Rouet et al., 2005). The low limit of detection for 75% of one spot of DBS was calculated by multiplying that of plasma by the correcting factor to obtain 2000 copies/ml or 3.30 log copies/ml (300 × 6.67) (Ou et al., 2007; Rouet et al., 2007; Viljoen et al., 2010).

#### 2.4.2. Generic HIV DNA cell kit

This kit is a derivative test of the previous one. The primers and the Taqman probes are the same (Schvachsa et al., 2007; Avettand-Fènoël et al., 2009; Avettand-Fénoël et al., 2008, 2009; Burgard et al., 2012). Detection and quantitation of HIV-1 DNA by real time PCR were carried out on cellular DNA extracted from 200 μl of cell pellet by QiaAmp DNA mini kit as previously described (Schvachsa et al., 2007; Avettand-Fènoël et al., 2009; Avettand-Fénoël et al., 2008, 2009; Burgard et al., 2012) and from one spot by the same kit as describe above. In quantitation methods, results might be standardised; DNA extracts were quantified using fluorescence readings at 260 nm by the Biophotometer (Eppendorf, Hamburg, Germany) and diluted in $H_2O$ to test 1 μg of total DNA per PCR, which was considered to be equivalent to 150,000 cells (Dib et al., 1996). The manufacturer supplied ready extracted quantitative standards to determine cellular DNA viral load based on 8E5 cell line (ATCC CRL 8993), containing a single integrated copy of HIV-1 proviral DNA per cell (Folks et al., 1986). The sensitivity threshold was 6 copies/PCR, equivalent to 40 copies/millions of cells, and to 1 μg of DNA (Avettand-Fénoël et al., 2008). ABI Prism 7000 SDS was used for both quantitative and qualitative methods.

#### 2.4.3. Amplicor HIV-1 DNA qualitative test v1.5

Qualitative detection of HIV-1 DNA by this kit is based on conventional PCR using two sets of primers specific to the DNA target, an Internal Positive Control (IPC) for the amplification and two specific probes for revelation by the colorimetric ELISA method as described elsewhere (Young et al., 2000; Kébé et al., 2011). Briefly, one spot was partially cut out and washed to collect leukocytes by centrifugation before lysis with detergent and Proteinase K at 60 °C for 30 min and at 100 °C for 30 min. A target 155-bp region of the gag gene was amplified simultaneously with an IPC on the Applied Biosystems GeneAmp 9600 PCR System (Applera International, Foster city, CA, USA). The master mix contained two biotinylated primer pairs specific for both HIV-1 DNA and IPC. The detection of amplified DNA was performed using target-specific oligonucleotide probes that allow the independent identification of the HIV-1 DNA and the IPC amplicons with an ELISA channel analyser containing a PW 40 plate washer [Bio Rad, Hercules, California, USA] and an EL 800 PC absorbance microplate reader [Bio Tek, Winoosky, Vermont, USA]. The cut off $OD_{450}$ was 0.2 for HIV-1 and IPC and interpretation was made according to the manufacturer's instructions (Kébé et al., 2011).

### 2.5. Statistical analysis

Diagnostic sensitivity and specificity, as well as positive and negative likelihood ratios of the Generic HIV-DNA Cell kit and Qualitative Amplicor HIV-1 DNA Test v1.5 on DBS and the Generic HIV Charges Viral kit on plasma and DBS were compared to the reference test and assessed. All viral load values were $\log_{10}$ transformed. To describe the concordance between the viral loads measurements obtained on plasma and those obtained on DBS, the linear regression method was used and Pearson's correlation was calculated. Agreement between both methods of measurement was assessed using the Bland–Altman method; a range of agreement was defined as mean bias ±2 SD (Bland and Altman, 1986). All analyses were performed using R statistical software including the *ResearchMethods* package.

### 2.6. Ethical aspects

The ethics committee of the Ivorian Ministry of Public Health approved the protocol in 2008 and a signed consent form was obtained from both the mother and father or from legal guardian(s) when exposure to HIV-1 was confirmed and before obtaining any blood sample from their child.

## 3. Results

Overall, over the study period, samples were collected from 223 children, 50 HIV-infected children, and 173 non-infected children (Fig. 1). Of all the associated DBS samples, 13 never arrived to the laboratory and one was not in line with the recommendations. Another additional 13 DBS samples were excluded from the analysis for poor or average quality. Two out of the HIV-infected children were already on ART and were excluded. At the end of this selection process, 138 children were included, 46 were HIV-infected and 92 non-infected.

### 3.1. Baseline characteristics

Baseline characteristics are described in Table 1. The median age at HIV-screening was higher in HIV-infected children (21 months, IQR: [12–28]) compared to those not HIV-infected (2 months, IQR: [1–12 months]) ($p = 0.0004$).

PMTCT interventions were frequent in both groups but the exposure to a maternal PMTCT prophylaxis was more frequent in HIV-infected children (89.1%) compared to non-infected children (64.1%), ($p = 0.00034$). Inversely, HIV-infected children were less frequently exposed to postnatal prophylaxis at six weeks compared to the non HIV-infected children (68.5% versus 36.9%, $p < 000.1$). More than 2/3 of the children were exposed to breastfeeding. There was no statistical difference in the feeding between HIV-infected and non-infected children.

The median HIV-DNA level on EDTA samples from infected children at diagnosis was 3.15 $\log_{10}/10^6$ cells [IQR: 2.70–3.37]. The median plasma and DBS HIV-RNA levels were respectively 5.82 $\log_{10}$ copies/ml (IQR: [5.23–6.57]) and 5.17 $\log_{10}$ copies/ml (IQR: [4.64–5.77]).

### 3.2. Diagnostic performances of the different tests

All four tests (Generic HIV Charges Viral kit on plasma, Generic HIV Charges Viral kit on DBS, Generic HIV DNA Cell kit on DBS and Amplicor and HIV-1 DNA Qualitative Test v1.5 on DBS) were 100% specific and 100% sensitive (Table 2) at the thresholds described in

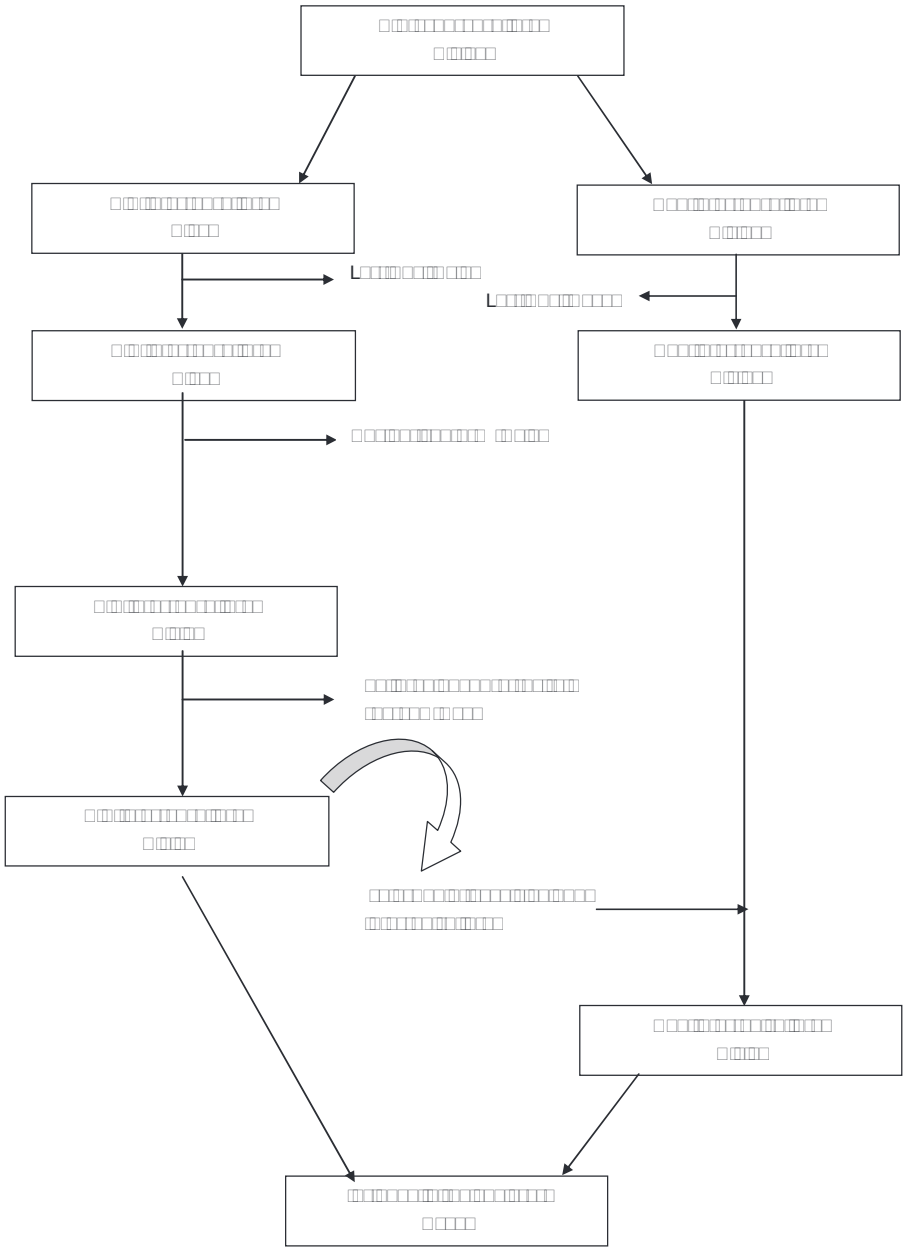

**Fig. 1.** Flow diagram of the 138 children included in the Pedi-Test-DBS ANRS 12183 Study, Abidjan, Côte d'Ivoire (March–September 2008).

the methods section. Consequently, these tests classify as excellent when diagnosing HIV in infants.

### 3.3. Correlation between HIV-RNA levels on plasma and on DBS

The median plasma and DBS HIV-RNA levels were 5.82 $\log_{10}$ copies/ml (IQR: [5.23–6.57]) and 5.05 $\log_{10}$ copies/ml (IQR: [4.52–5.65]) respectively; Pearson's correlation coefficient was $R^2 = 0.92$ statistically significant ($p < 0.0001$) (Fig. 2a). Viral loads were higher in plasma than those measured on DBS samples. The differences ranged from −0.52 to 1.11 $\log_{10}$ copies/ml, and the mean bias was 0.65 (SD = 0.35). The Bland–Altman analysis (Fig. 3) indicated that the 95% limits of agreement between the two methods ranged from −1.35 to 0.06. Only one result was out of these limits; it corresponded to one child who had plasmatic RNA viral load of 2.78 $\log_{10}$ versus a viral load on DBS of 3.30 $\log_{10}$.

### 3.4. Correlation between HIV-1 DNA on cell pellets and HIV-1 RNA level on plasma and DBS

The median HIV-1 DNA on EDTA cell pellets was 3.15 $\log_{10}/10^6$ cells [IQR: 2.70–3.37].

As presented in Fig. 2b and c, there was good correlation between HIV-DNA on EDTA cell pellets and plasma HIV-RNA levels (Pearson's correlation coefficient $R^2 = 0.56$, ($p = 0.0003$) and on DBS HIV-RNA levels (Pearson's correlation coefficient $R^2 = 0.51$, $p < 0.0001$).

## 4. Discussion

This study, performed in field conditions, compared both the diagnostic performances of PCR testing for HIV-RNA on EDTA blood plasma and on DBS with HIV-DNA and their respective levels on child samples in Abidjan, Côte d'Ivoire. Both tested methods had identical diagnostic performances; sensitivity and specificity for

**Table 1**
Baseline characteristics of the 138 children included in the Pedi-Test-DBS ANRS 12183 Study. Abidjan, Côte d'Ivoire, 2008.

| | HIV-infected $N=46$ | Non-infected $N=92$ | $P$ |
|---|---|---|---|
| Age at diagnosis (months)* | 21 [12–28]** | 2 [1–12] | 0.0004 |
| Mother's antiretroviral prophylaxis (PMTCT) | | | 0.0034 |
| Yes | *41 (89.1)*** | *59 (64.1)* | |
| No | *1 (2.2)* | *21 (22.8)* | |
| Unknown | *4 (8.7)* | *12 (13.1)* | |
| Child's antiretroviral prophylaxis (PMTCT) | | | <0.0001 |
| Yes | 17 *(36.9)* | 63 *(68.5)* | |
| No | 26 *(56.5)* | 17 *(18.5)* | |
| Unknown | 3 *(6.6)* | 12 *(13)* | |
| Breastfeeding modalities | | | 0.1966 |
| Exclusive | 33 *(71.7)* | 59 *(64.1)* | |
| Mixed | 4 *(8.7)* | 3 *(3.3)* | |
| Formula fed | 4 *(8.7)* | 19 *(20.7)* | |
| Unknown | 5 *(10.9)* | 11 *(11.9)* | |

* For children with available data, $N=122$; PMTCT: prevention of mother-to-child prophylaxis.
** Interquartile range.
*** Numbers in parenthesis are percentages.

early infant diagnosis were both 100%. However, viral load levels on plasma and DBS were not consistently at the same level.

In this study, a small proportion of children was excluded because their DBS samples were not collected (2.7%). Lack of information among the healthcare workers of the involved clinical sites and logistical inability to trace children after they left the clinics were the common reasons. Among the collected DBS samples, a few were rejected (2.7%) for poor or average quality at the beginning of the study. After two staff training sessions within the clinical sites, all the DBS samples were of good quality. Overall 26 DBS samples (5.4%) could not be analysed. Globally, collecting DBS samples is feasible with training and assistance during the early period of DBS implementation to guarantee that >90% of DBS samples are taken from the clinical sites to the laboratory. All DBS samples were received within the 15-day period recommended by the Ivorian National Programme for HIV care (data not shown)

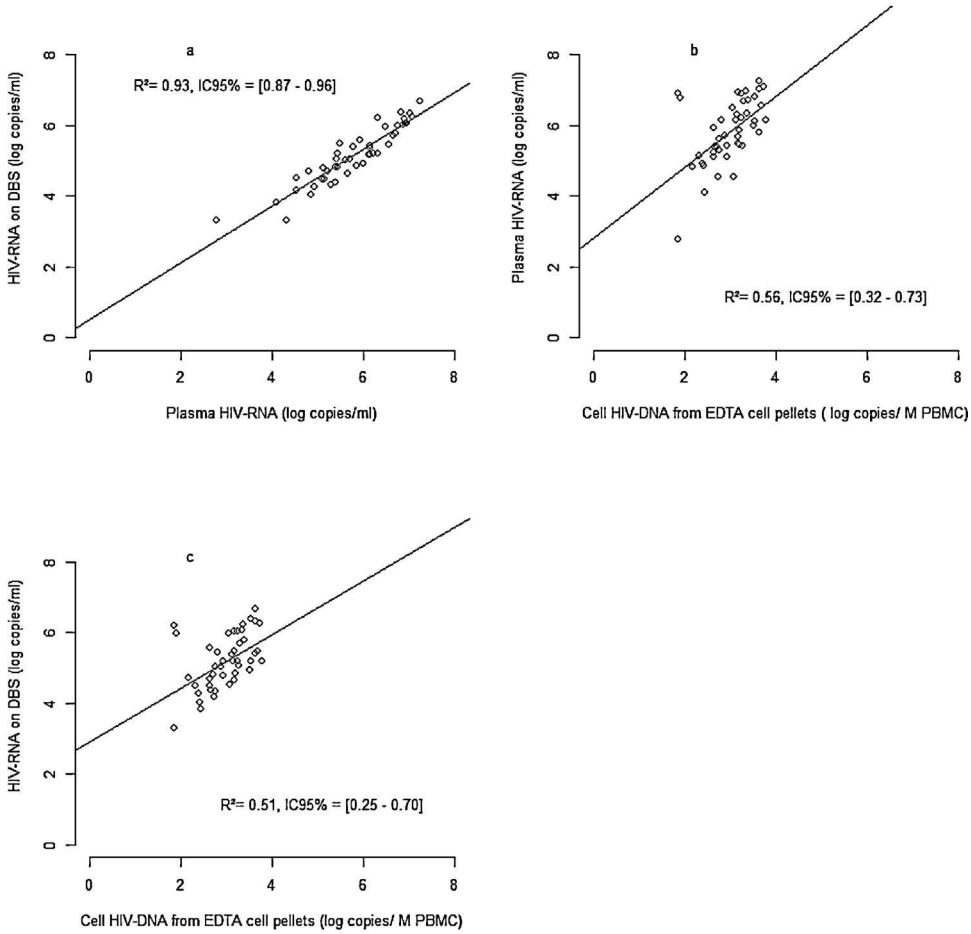


**Fig. 2.** Correlation between respectively (a) HIV RNA on plasma and DBS, (b) cell HIV DNA on EDTA cell pellet and HIV RNA on DBS, and (c) cell HIV DNA on EDTA cell pellet and HIV RNA on plasma, in The Pedi-Test DBS ANRS 12183 Study, Abidjan, Côte d'Ivoire, 2008. $N=46$ HIV-1 infected children.

**Table 2**
Comparison of the four tests to reference standard test, Generic HIV DNA Cell kit (Biocentric) on whole blood in the 138 children of the Pedi-Test-DBS ANRS 12183 Study, Abidjan, Côte d'Ivoire, 2008.

| | Reference test Generic HIV DNA Cell kit (Biocentric) on whole blood | | | |
|---|---|---|---|---|
| | Positive | Negative | Total | |
| Generic HIV DNA Cell kit (Biocentric) on DBS | | | | |
| Positive | 46 | 0 | 46 | Sensitivity 100% |
| Negative | 0 | 92 | 92 | Specificity 100% |
| Total | 46 | 92 | 138 | |
| Roche Amplicor HIV-1 DNA Test v1.5 (Roche Diagnostics) on DBS | | | | |
| Positive | 46 | 0 | 46 | Sensitivity 100% |
| Negative | 0 | 92 | 92 | Specificity 100% |
| Total | 46 | 92 | 138 | |
| Generic HIV RNA Charge Virale Cell Kit (Biocentric) on plasma | | | | |
| Detectable | 46 | 0 | 46 | Sensitivity 100% |
| Undetectable | 0 | 92 | 92 | Specificity 100% |
| Total | 46 | 92 | 138 | |
| Generic HIV RNA Charge Virale Cell Kit (Biocentric) on DBS | | | | |
| Detectable | 46 | 0 | 46 | Sensitivity 100% |
| Undetectable | 0 | 92 | 92 | Specificity 100% |
| Total | 46 | 92 | 138 | |

(Public Health Ministry of Cote d'Ivoire, 2006). In a context where PMTCT interventions are scaling-up, the maternal and child PMTCT described in this study were high (>80%) in both infected children and uninfected children compared to previous data published in Côte d'Ivoire (Anaky et al., 2010; Stringer et al., 2010). These findings could suggest that some mothers did not complete the PMTCT process for themselves and their child as described in other studies (Anaky et al., 2010; Stringer et al., 2010). This emphasizes the need for efforts to strengthen the link between PMTCT and child-care programmes and to respond to maternal non-adherence (The KIDS-ART-LINC Collaboration, 2008; Anaky et al., 2010; Stringer et al., 2010).

We did not study the level of HIV DNA detected in DBS and therefore were not able to study the correlation between HIV DNA level quantified on whole blood and DBS. However, the two

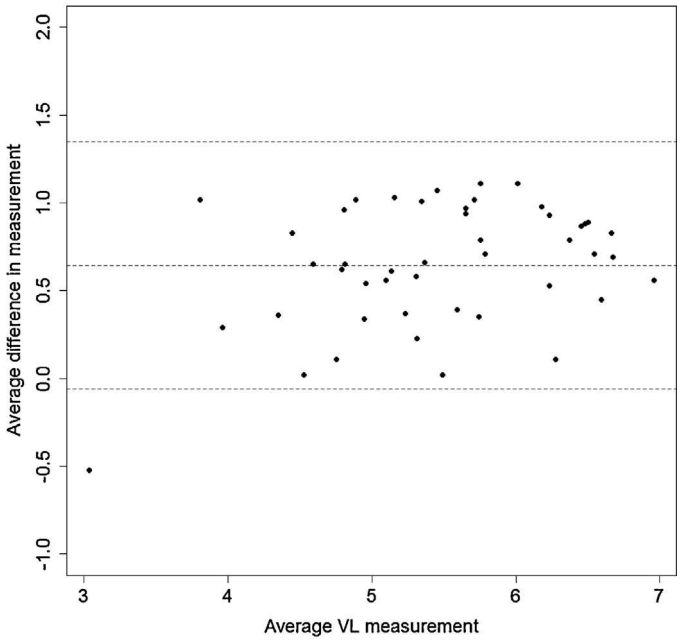


**Fig. 3.** Bland–Altman analysis of agreement between HIV RNA plasma and DBS in the Pedi-Test-DBS ANRS 12183 Study, Abidjan, Côte d'Ivoire. The horizontal lines represent the mean difference and ±2 standard deviation.

Biocentric assays performed well for HIV-1 infant diagnosis on whole blood collected on EDTA tubes and on DBS paper for HIV-1 infant diagnosis with 100% specificity and sensitivity. These findings support others made using the same kits in Burkina Faso and South Africa (Viljoen et al., 2010). Compared to the main competing test, the Qualitative Amplicor HIV-1 DNA test v1.5 on DBS, these kits showed very good performances for both HIV-DNA and HIV-RNA detection. At diagnosis, the median cellular HIV-1 DNA viral load was high (3.15 $\log_{10}$ copies/$10^6$ cells) and consistent with data described from a Northern cohort comprised of infants aged 1–6 months (3.0–3.9 $\log_{10}$ copies/$10^6$ cells) (Burgard et al., 2012). Cell HIV-1 DNA levels were highly correlated with HIV-1 RNA levels measured in plasma and DBS. These findings could be explained by the natural history of HIV-1 infection in children, characterised by high levels of plasmatic HIV-1 RNA viral load and rapid progress to AIDS and death in a context where PMTCT programmes lack performance (Stringer et al., 2010).

HIV-1 RNA levels measured on plasma and DBS were strongly correlated which is consistent with others studies that have shown a high correlation between HIV-1 RNA levels on plasma and DBS with the Biocentric kit and other commercial kits (Johannessen et al., 2009; Lofgren et al., 2009; Reigadas et al., 2009; Viljoen et al., 2010; Kébé et al., 2011). The median plasmatic viral load level was in line with values obtained in other studies (5.1–5.6 $\log_{10}$ copies/ml) (Rouet et al., 2005, 2007; Viljoen et al., 2010; Kébé et al., 2011; Burgard et al., 2012). However, HIV-RNA levels on DBS were underestimated compare to those found on blood plasma; the mean difference was 0.65 $\log_{10}$ copies/ml ranging from −0.52 to 1.11 $\log_{10}$. It is possible that the assumption to calculate the amount of blood explored on DBS might be underestimated. Another limit of these results is due to the fact that the HIV-RNA tests were done with the objective of infant diagnosis and DNAse was not apply on extracts, the quantification included both HIV-RNA as well as HIV-DNA levels. The extraction method used during this study is a plausible explanation of this observation: although it was cheaper it had disadvantages such as being manual. A new small nucleic acid extractor that rends the technique easier and more feasible was recently tested (data not shown). A previous study has shown that the extraction method used in DBS testing is a critical factor in obtaining reliable results (Monleau et al., 2009). Indeed, the authors compared four available methods and found that in terms of efficiency, the ranking was as follows: Nuclisens kits > Abbott sample preparation system > Roche high pure viral nucleic acid kit > Qiagen QIAamp viral RNA mini kit. But, using Nuclisens Mini Mag (manual) or Easy Mag (automatic) or modified QIAamp Viral RNA mini kit extraction, other authors showed good correlation and little bias between plasmatic and DBS viral load with the Generic HIV Charge Viral kit (Monleau et al., 2009; Reigadas et al., 2009; Viljoen et al., 2010). However Nuclisens assays are expensive and need a semi automatic or automatic device for implementation. And the modified QIAamp Viral RNA mini kit method needs some additional reagents and steps. Clinically, the important differences observed between HIV-RNA levels on plasma and DBS are not compatible with interchanging both screening methods (plasma and DBS) However, the high level of Pearson's correlation indicates that protocol could be adapted to improve the results, including testing a higher volume of blood.

## 5. Conclusion

The performances of the Biocentric assays on DBS for routine HIV diagnosis in children were satisfactory showing that these assays are feasible, and that is crucial to improve early access to ART in infants on a large scale in low income countries. This first serial

of results shows that DBS could also provide an initial viral load measurement for follow-up to facilitate viral load monitoring.

## Sources of support

This study was supported by the French Agency for Research on AIDS and viral Hepatitis (ANRS), grant ANRS 12183. Sophie Desmonde is a fellow of the Ecole des Hautes Etudes en Santé Publique (EHESP), Rennes, France. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

## Conflict of interest

The authors declare not conflicts of interest.

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