International Journal of Infectious Diseases 111 (2021) 253–260

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

HIV-1 diagnosis using dried blood spots from patients in Kinshasa, DRC: a tool to detect misdiagnosis and achieve World Health Organization 2030 targets

David Barquín[a], Adolphe Ndarabu[b], Silvia Carlos[c,d,e], Mirian Fernández-Alonso[a,c,d], Marina Rubio-Garrido[f], Benit Makonda[b], África Holguín[f], Gabriel Reina[a,c,d,*]

[a] Microbiology Department, Clínica Universidad de Navarra, Pamplona, Spain
[b] Centre Hospitalier Monkole, Kinshasa, Democratic Republic of the Congo
[c] ISTUN, Institute of Tropical Health, Universidad de Navarra, Pamplona, Spain
[d] IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
[e] Department of Preventive Medicine and Public Health, Universidad de Navarra, Pamplona, Spain
[f] HIV-1 Molecular Epidemiology Laboratory, Microbiology and Parasitology Department, University Hospital Ramón y Cajal-IRYCIS and CIBEREsp-RITIP, Madrid, Spain

ARTICLE INFO

Article history:
Received 5 April 2021
Revised 16 August 2021
Accepted 17 August 2021

KEYWORDS:
HIV-1
Dried blood spots (DBS)
Rapid diagnostic test (RDT)
Sub-Saharan Africa (SSA)
Misdiagnosis
Democratic Republic of the Congo (DRC)

ABSTRACT

Introduction: Currently, only 54% of the population of the Democratic Republic of the Congo (DRC) know their HIV status. The aim of this study was to detect HIV misdiagnosis from rapid diagnostic tests (RDT) and to evaluate serological immunoassays using dried blood spots (DBS) from patients in Kinshasa, DRC.
Methods: Between 2016 and 2018, 365 DBS samples were collected from 363 individuals and shipped to Spain. The samples were from people with a new HIV positive (n = 123) or indeterminate (n = 23) result, known HIV-positive patients (n = 157), and a negative control group (n = 62). HIV serology was performed using Elecsys HIV combi PT (Roche), VIDAS HIV Duo Quick (BioMérieux), and Geenius (Bio-Rad). In addition, HIV RNA detection was performed in all samples using the COBAS AmpliPrep/COBAS Taqman HIV-1 Test 2.0 (Roche).
Results: Overall, 272 samples were found to be positive and 93 to be negative for HIV serology. The sensitivity was 100% for both Elecsys and VIDAS techniques, but specificity was slightly higher for the VIDAS test: 100% (96.1–100%) vs 98.9% (94.1–99.9%). Of the 23 indeterminate cases using RDT, only three cases were true-positives with a detectable viral load. Eleven samples out of the 280 classified as positive by RDT corresponded to nine patients who had received a false diagnosis of HIV through RDT (3.9%); six of them had been on antiretroviral therapy for at least 2 years.
Conclusions: Elecsys HIV combi PT and VIDAS HIV Duo Quick immunoassays showed high sensitivity and specificity when using DBS. RDT-based serological diagnosis can lead to HIV misdiagnosis with personal and social consequences in sub-Saharan Africa.

Introduction

The Joint United Nations Programme on HIV/AIDS (UNAIDS) 95–95–95 targets to end the AIDS epidemic propose that 95% of all people living with HIV should know their HIV status by 2030, 95% of those diagnosed should receive antiretroviral therapy (ART), and 95% of them should be virally suppressed (Gisslén et al., 2017, Guichet et al., 2016). Differences in treatment algorithms and prevention strategies between different sub-Saharan Africa (SSA) regions and risk groups has led to variable global and regional HIV incidence and prevalence (Hemelaar et al., 2019, Rubio-Garrido et al., 2020, Rubio-Garrido et al., 2019). Currently, 93% of people infected with HIV live in low-income countries (De Mulder and Holguín, 2013, UNAIDS. UNAIDS 2020), almost 70% in SSA. These countries harbour a high number of different circulating HIV-1 variants and have limited access to routine HIV monitoring (Guichet et al., 2016, Rubio-Garrido et al., 2019). The global number of new HIV infections declined between 2010 and 2017, and

* Corresponding author: Gabriel Reina, Microbiology Department, Clínica Universidad de Navarra, Pamplona, Spain. Tel: +34-948-255400 ext. 5103
E-mail address: gabi@unav.es (G. Reina).

https://doi.org/10.1016/j.ijid.2021.08.035

SSA had the leading role in this reduction in new HIV infections (UNAIDS. UNAIDS 2020). However, the implementation of the previous 90–90–90 targets for 2020 in the West and Central Africa regions has been slow, with 68% of people currently knowing their HIV status, 85% of whom are on treatment and 45% of viral suppression among people living with HIV. In the Democratic Republic of the Congo (DRC), 54% of people living with HIV know their HIV status, 49% of them receive treatment, and there are no data about the third target (viral suppression) (UNAIDS. UNAIDS 2020).

In the DRC, genetic variability of HIV is increasing, especially the number of circulating complex recombinants (Hemelaar et al., 2019), which could be associated with misdiagnosis (Vidal et al., 2000, Shanks et al., 2013, Kufa et al., 2017). The World Health Organization (WHO) has proposed a diagnosis algorithm based on three consecutive HIV rapid diagnostic tests (RDTs) and/or enzyme immunoassays (EIAs) in countries with a prevalence below 5% with an affordable price. Three reactive tests are required for a positive diagnosis, but if the third RDT is negative, the patient is classified as having HIV-inconclusive results (World Health Organization, 2019). This approach appears to be appropriate for use in regions with minimal infrastructure and training, as no expensive equipment or laboratory facilities are required. In addition, it enables HIV testing services to be delivered in non-laboratory settings (World Health Organization 2019). However, some studies have suggested that data from local evaluations are important for assessing diagnostic accuracy in the specific setting (Do Rosario Augusto et al., 2020).

RDTs can use fourth-generation techniques, detecting both HIV antibodies and p24 antigen simultaneously. The drawback of RDTs is their subjective reading, which can raise doubts regarding the interpretation of the results, and they do not appear to be as sensitive as the standard combined test (HIV Combo assay) performed using automated high-throughput instruments. The ability of the HIV Combo assay to detect HIV antigen empowers the test with the capacity to detect recent HIV infections during the antibody-negative window period, increasing the sensitivity and reducing the detection of false-negatives. RDT-based algorithms have enabled the implementation of HIV programmes and surveillance in resource-limited settings and they have been crucial to scaling up access to life-saving treatment. However, there are also cases of HIV misdiagnosis with these test algorithms. These false diagnosis cases are a risk for the HIV programmes in resource-limited countries. They have been reported and attributed to a variety of factors, including cross-reactivity, misinterpretation, HIV variability, administrative errors, RDT storage and transport conditions, lack of staff training, sample origin, the patient's sex, comorbidities, and other factors associated with the geographic region (Hemelaar et al., 2019, Shanks et al., 2013, Kufa et al., 2017, Kosack et al., 2017, Klarkowski et al., 2014). An alternative to the traditional biological samples used for diagnosis (plasma or whole blood) are dried blood spots (DBS). DBS are cheaper, simple to store and transport, allow further molecular testing, and use a minimum volume of blood, making them ideal for use in resource-limited countries (De Mulder and Holguín, 2013). DBS have been demonstrated to be an alternative for HIV diagnosis instead of plasma and have been used with standard HIV immunoassays in centralized facilities. Automated immunoassays performed on DBS can be more efficient than RDTs (Tuaillon et al., 2020). Nevertheless, they can have lower sensitivity than serum/plasma due to the lower concentration of antigen/antibodies or a delay in diagnosis.

The aim of this study was to detect HIV misdiagnosed cases by comparing the results of the RDT-based HIV testing algorithm currently used in DRC with the analysis of collected DBS samples tested on centralized HIV testing platforms.

Materials and methods

Study design and participants

The OKAPI project (Observational Kinshasa AIDS Prevention Initiative) is a prospective cohort study designed to evaluate factors associated with changes in HIV knowledge and sexual behaviours after 6 and 12 months of follow-up. From April 2016 to April 2018, people aged 15–59 years attending HIV voluntary counselling and testing (VCT) at Monkole Hospital Centre in Kinshasa and patients coming from Hospital Kalembelembe were invited to participate.

For this specific study, samples were collected from participants who received a new HIV positive (n = 123) or indeterminate result (n = 23) within the OKAPI project, which were tested together with a group of samples from known HIV-positive patients (n = 157) and an HIV-negative control group (n = 62).

Blood sample collection

Specimens from the OKAPI project (n = 146), all negative controls (n = 62), and samples from a subset of HIV-infected patients not on ART (n = 23) were collected simultaneously for RDT and DBS. Samples from HIV-positive individuals on ART (n = 134) were exclusively collected to prepare DBS, since the patients had been diagnosed previously.

Samples were collected by venipuncture for RDT analyses and 70 µl of whole blood was placed with a micropipette onto each spot on a Whatman Paper 903 Protein Saver Card (Schleiche and Schuell, USA). Each card contained five dots. Two or three cards were collected for each participant. They were dried separately on a drying-rack overnight at room temperature at Monkole and Kalembelembe hospitals (Kinshasa, DRC), sealed in a zip-lock plastic bag with desiccant bags and stored at −20°C until shipped in dry ice to Clínica Universidad de Navarra (Pamplona, Spain), where they were stored at −80°C until further use. A total of 365 samples were collected for this study and analysed in advance in Kinshasa.

Anti-HIV laboratory analysis

HIV serological diagnosis in Kinshasa (DRC) was performed using the following RDTs (immunoassays): Alere Determine HIV-1/2 Ag/Ab (Abbott), Double-Check Gold HIV 1&2 (Orgenics), and Uni-Gold HIV (Trinity Biotech). The algorithm used for the diagnosis of HIV infection in Kinshasa during 2016–2018 consisted of one positive result with the Abbott test as screening and then two additional RDTs performed for confirmation. If two RDT results are positive, the sample is classified as positive. In the case of discordant results, where the initial Abbott screening test is positive, but both confirmatory RDTs are negative, the sample is classified as an 'indeterminate' result requiring further testing. In that case, after 2 weeks the patient is scheduled to return for a second confirmatory testing using RDT.

At the laboratory of the Clínica Universidad de Navarra in Spain, the blood was extracted from one dot of DBS by elution in 1 ml of phosphate buffered saline (PBS), for 60 minutes at 37°C. The samples were analysed with two immunoassay tests: Elecsys HIV combi PT (Roche) and VIDAS HIV Duo Quick (BioMérieux), both fourth-generation assays. The Elecsys HIV combi PT (Roche) is an in vitro electrochemiluminescence immunoassay test for qualitative detection of anti-HIV-1 and anti-HIV-2 antibodies and p24 HIV-1 antigen simultaneously, for which the cut-off index (COI) is 1.00. The VIDAS HIV Duo Quick (BioMérieux) is a test that combines also two enzyme immunoassay reactions for the detection of p24 antigen and anti-HIV-1 and anti-HIV-2 antibodies; the cut-

off test value (VT) of this test is 0.25. The assay takes 80 minutes, compared to 27 minutes for the Elecsys HIV combi PT (Roche). The sample volume used is 200 µl for VIDAS and 40 µl for Elecsys.

Finally, 135 randomly selected samples were subjected to further serological testing and differentiation of either HIV-1 or HIV-2 antibodies. For this purpose, 40 µl of the previous DBS eluted product was used to perform the Geenius HIV 1/2 confirmatory assay (Geenius, Bio-Rad, USA), according to previous reports using DBS (Fernández McPhee et al., 2015). For the interpretation of the results, the intensity of the band of each antigen that appeared in the assay was classified into three categories: non-reactive (no band appeared), low intensity (band appeared with low intensity or colour), high intensity (clear colour of the band appeared).

HIV seropositive patients (true-positive) were considered those with at least two positive tests among the three techniques used from DBS (Elecsys, VIDAS, and Geenius). This criterion was used for sensitivity and specificity calculation.

HIV-1 viral load

The HIV-1 viral load was analysed in all samples (n = 365) using the COBAS AmpliPrep/COBAS Taqman HIV-1 (Roche). A new DBS dot was eluted with 1.2 ml of specimen pre-extraction reagent (SPEX; Roche, Mannheim, Germany) for 10 minutes at 56°C. The number of HIV-1 RNA copies per dot and per millilitre of plasma was obtained, after considering the patient's haematocrit, assuming a haematocrit of 42% for females and 47% for males according to previous studies from SSA (Rubio-Garrido et al., 2019, Miri-Dashe et al., 2014). This led to plasma volumes of 40.6 µl and 37.1 µl, respectively, in 70 µl blood collected per dot.

Ethical issues

The project was approved by the human subjects review committees at Monkole Hospital/University of Kinshasa (Kinshasa, DRC) and University of Navarra (Pamplona, Spain). Informed consent was obtained from the enrolled participants. All methods were performed in accordance with relevant guidelines and regulations.

Statistical analysis

All statistical analyses were conducted in Stata 15.0 statistical program. A descriptive analysis was performed to examine demographic and clinical characteristics and ART data. The mean, median, and interquartile range (IQR) were calculated for quantitative variables and percentages were analysed for qualitative variables.

Results

From April 2016 to April 2018, a total of 365 DBS specimens were collected. Out of the 365 study samples, 303 (83%) yielded a positive (n = 280) or indeterminate (n = 23) result using RDTs in the DRC and 62 (17%) were negative (Figure 1).

The majority of patients enrolled in the study were female (64% female, 36% male). The mean age of participants was 41.4 years (range 15–59 years) and the median CD4 count was 264/µl (IQR 152–427/µl).

Regarding antiretroviral treatment, among 280 HIV-positive patients locally, 157 (56%) were receiving ART, 108 (39%) had never received treatment, and no data were available for the remaining 15 (5%) patients due to loss to follow-up. The regimens were based almost exclusively on nucleoside reverse-transcriptase inhibitors (NRTIs) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs); the most commonly used combination was zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP) as first-line therapy (76 patients) and tenofovir disoproxil fumarate (TDF) + 3TC + efavirenz (EFV), mainly as alternative treatment (25 patients).

As shown in Table 1, a total of 365 DBS samples were tested simultaneously with Elecsys and VIDAS serological assays. The Elecsys HIV combi PT (Roche) test was positive for 273 (74.8%) DBS samples (median COI 268, IQR 112–763) and negative for 92 samples (25.2%) (median COI 0.234, IQR 0.203–0.287). The VIDAS HIV Duo Quick (BioMérieux) resulted positive for 272 (74.5%) DBS samples (median VT 20.2, IQR 14.6–25-4), while 93 samples (25.5%) were negative (median VT 0.16, IQR 0.15–0.17).

The diagnostic accuracy of the HIV serological screening assays and the total adjusted sensitivity and specificity of both immunoassays were close to 100%. However, the specificity of the Elecsys HIV combi PT (Roche) was lower (98.9%) due to a false-positive result. Regarding the Geenius HIV 1/2 confirmatory assay (Geenius, Bio-Rad, USA), 135 (36.9%) out of the 365 collected DBS were randomly selected and tested. Among them, 100 (74.1%) gave a positive HIV-1 diagnosis mainly due to the presence of antibodies against gp-160 and gp-41 antigens, and 35 (25.9%) were negative or indeterminate. Among the 100 HIV-1-positive samples, 100% were positive by Elecsys HIV combi PT (Roche) and VIDAS HIV Duo Quick (BioMérieux). However, among the 35 HIV-1-negative/uncertain samples, nine (25.7%) were simultaneously HIV-1-positive by Elecsys HIV combi PT (Roche) (COI range 3.35–45.54) and VIDAS HIV Duo Quick (BioMérieux) (VT range 0.48–12.12); in addition, two of them had detectable HIV-1 RNA. All of the indeterminate samples by Geenius testing (5) were found among these nine samples, due to the unique presence of a low intensity band in the gp-41 line in all of them. No HIV-2 infection was detected.

Table 2 shows in detail the data of the nine participants studied in this series who had a corresponding HIV misdiagnosis in Kinshasa. One of them (CUN182), a 31-year-old male misdiagnosed in 2016, was studied at three different points with three different DBS samples obtained. As shown, most patients were receiving ART when the DBS sample collection was carried out. Among these samples, only one demonstrated a positive result in one of the three immunoassays (Elecsys); however, it tested HIV-negative by VIDAS and Geenius, while the viral load was undetectable. Therefore, the Elecsys result was considered a false-positive, resulting in a lower specificity than the remaining techniques (Table 2).

In addition, of 23 participants with inconclusive RDT HIV results, three had HIV RNA detected in DBS and were considered as HIV-infected, and 20 participants were considered HIV-uninfected (Table 3).

All samples were analysed by COBAS AmpliPrep/COBAS for the detection and quantification of HIV-1 RNA as an additional confirmatory assay and to rule out acute infections. Viral load results within the quantification range of the assay were available for 232 (63.5%) of 365 samples, but HIV-1 RNA was not detected in 133 (36.4%) samples. The median viral load in the positive samples was 1225 copies/ml (IQR 562–33 916 copies/ml). After eliminating data from individuals on ART or unknown therapy, the sensitivity and specificity for HIV diagnosis by HIV RNA detection using DBS was 97.2% (95% CI 90.2–99.7%) and 98.9% (95% CI 92.3–99.9%), respectively.

Discussion

This study revealed 32 patients who had received an incorrect HIV diagnosis on site and had even started ART. In addition, the utility of DBS for serological and molecular analysis to confirm or rule out HIV infection was evaluated. HIV misdiagnosis can give rise to serious public health and individual consequences, not only in low-income countries (LICs) but also in high-income countries (Kosack et al., 2017, Lasry et al., 2019). These errors in diagnosis can decrease the validity of HIV diagnostic techniques, test-

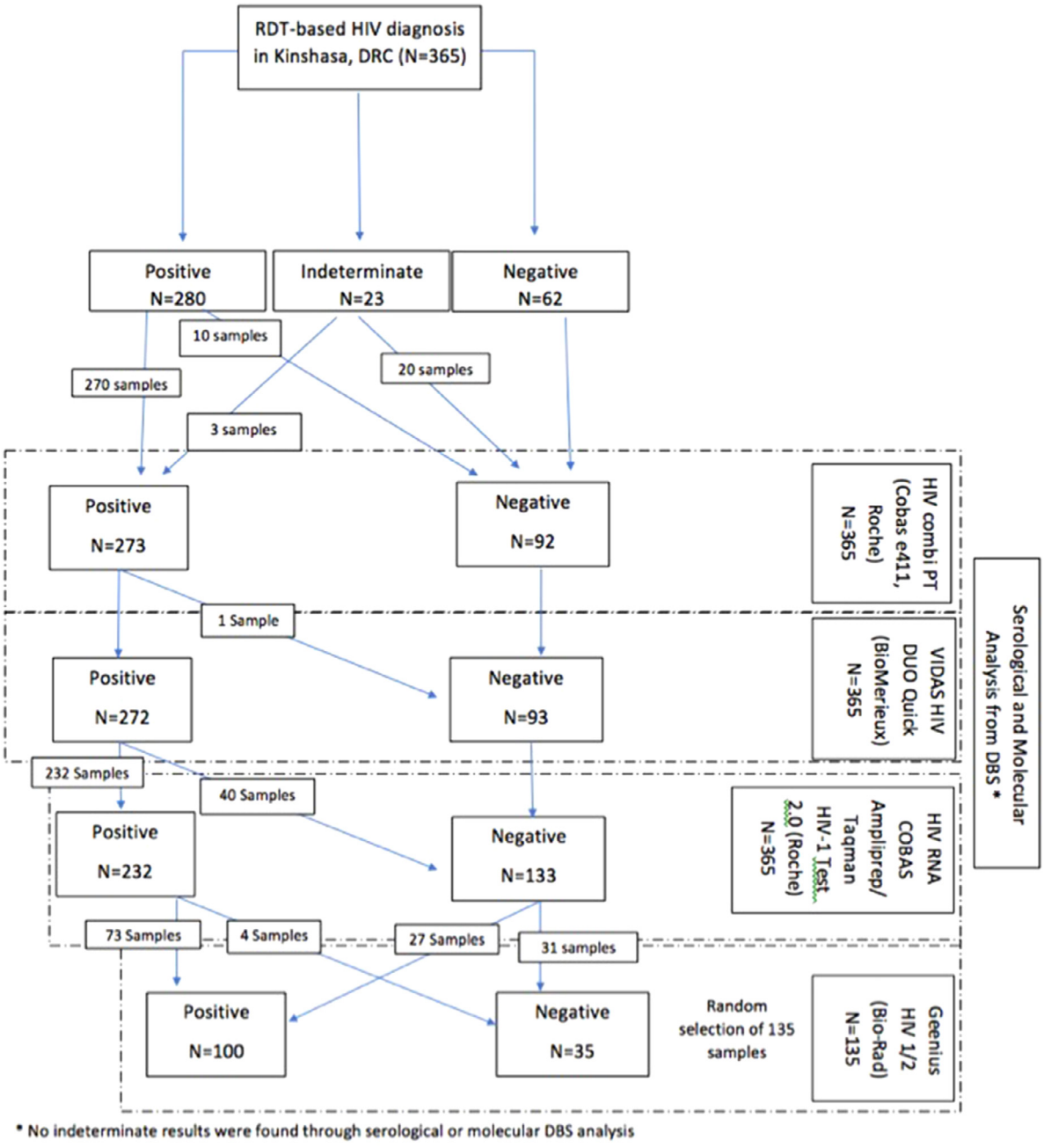

Figure 1. Summary of HIV diagnosis through RDT and DBS analysis for patients seen in Kinshasa (DRC).

ing programs and strategies, and increase the cost in patients who do not need ART. Currently, the WHO recommends retesting each patient to verify HIV infection before ART initiation (Lasry et al., 2019, Eaton et al., 2017). Thus, in LICs there is an imperative need to improve correct diagnosis. Moreover, the effects of an error in HIV testing, misclassification, and treatment of HIV-negative people has important health system consequences mainly in these countries. The use of DBS has been proposed as an alternative sample to plasma/serum that is easier to collect, store, and ship, and is very convenient in limited resource countries (Rubio-Garrido et al., 2019, De Mulder and Holguín, 2013, Fernández McPhee et al., 2015, WHO. Implementing HIV Viral Load Testing. 2014) for HIV testing and surveillance (Stefic et al., 2020). In this study, all samples were sent on DBS cards, allowing a cheap and easy way to transport them from DRC to Spain. The results obtained from RDT and DBS samples from HIV-infected patients were compared in virological failure, naïve subjects, and non-infected individuals.

RDTs have been proposed and recommended by the WHO for LICs due to their cost and the non-availability of other techniques; they recommend three consecutive positive RDTs in low prevalence settings (Eaton et al., 2017) like DRC with an estimated prevalence below 1%. The performance of RDTs within a WHO-recommended testing strategy and a validated testing algorithm has been shown to be highly reliable in different countries and settings in Africa (Dupwa et al., 2019), but there is a need to design accurate testing algorithms specific for the region. In addition, countries and administrations should strongly consider routine retesting before ART initiation (WHO recommendation) to avoid misdiagnosis and unnecessary treatment (Kosack et al., 2017), as well as the psychological trauma and stigmatization associated with HIV infection

Table 1
Summarized results for HIV diagnosis in Spain on Kinshasa (DRC) samples, through analytical assays for HIV diagnosis on dried blood spots. Sensitivity, specificity, PPV, and NPV (with 95% confidence interval) for Elecsys HIV combi PT, VIDAS HIV Duo Quick, and Geenius HIV 1/2 confirmatory assay.

		Elecsys HIV combi PT (Roche) ($n = 363$)		VIDAS HIV Duo Quick (BioMérieux) ($n = 363$)		Geenius HIV 1/2 confirmatory assay (Geenius, Bio-Rad, USA) ($n = 135$)[a]	
	Positive	Negative	Positive	Negative	Positive	Negative	
RDT ($n = 363$)	Positive ($n = 278$)	270	8	269	9	97	15
	Indeterminate ($n = 23$)	3	20	3	20	3	20
	Negative ($n = 62$)	0	62	0	62	0	0
Sensitivity		100% (98.6–100%)		100% (98.6–100%)		91.7% (84.9–96.1%)	
Specificity		98.9% (94.0–99.9%)		100% (96.0–100%)		100% (86.8–100%)	
PPV		99.6% (97.9–99.9%)		100% (98.6–100%)		100% (96.4–100%)	
NPV		100% (96.0–100%)		100% (96.0–100%)		74.3% (56.7–87.5%)	

NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test.
[a] Geenius HIV 1/2 assay evaluated using a random selection of 135 samples (see Figure 1).

Table 2
Characteristics and detailed testing results of participants with a false-positive local misdiagnosis by local RDT ($n = 9$).

	Subject data						Serology			RNA
ID	Date of HIV diagnosis	Age (years)	Sex	CD4 (cells/μl)	ART	ART duration (years)	HIV serology Elecsys Roche	HIV serology VIDAS BioMérieux	Geenius HIV 1/2 Bio-Rad	Viral load Roche
CUN41	2007	Unknown	Unknown	Unknown	Yes	11.9	Neg	Neg	Not done	Neg
CUN84	1992	27	M	234	Yes	19.2	Positive (COI: 2.05)	Neg (VT: 0.17)	Neg (no bands)	Neg
CUN99	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Neg	Neg	Neg	Neg
CUN117	2016	30	M	669	Yes	4.5	Neg	Neg	Not done	Neg
CUN130	Unknown	45	M	268	Yes	5.3	Neg	Neg	Not done	Neg
CUN136	2017	69	F	898	Unknown	Unknown	Neg	Neg	Not done	Neg
CUN182	2016	31	M	1029	Yes	4.4	Neg	Neg	Neg	Neg
CUN200	2012	62	F	436	Yes	4.2	Neg	Neg	Not done	Neg
CUN345	2012	32	F	Unknown	Unknown	3.2	Neg	Neg	Neg	Neg

ART, antiretroviral therapy; COI, cut-off index; F, female; M, male; RDT, rapid diagnostic test; VT, test value.

Table 3
Characteristics and detailed testing results of participants with an HIV indeterminate result by local RDT (n = 23).

Subject data							DBS serology				DBS molecular test	
Number	RDT-based HIV diagnosis	Date of HIV diagnosis	Age (years)	CD4 (units)	ART	ART duration (months)	Elecsys Roche (COI)	VIDAS BioMérieux (VT)	Geenius HIV 1/2 confirmatory assay Bio-Rad[a]	Viral load Roche (log)	Final diagnosis
20	Indeterminate	2016–2018	39.1 (SD 13.01)	Unknown	None	Unknown	Negative Median COI: 0.291 (IQR 0.24–0.31)	Negative Median VT: 0.16 (IQR 0.15–0.172)	gp36: – gp140: – p31: – gp160: – p24: – gp41: –	Not detected	HIV-1 negative
1	Indeterminate	Unknown	24.9	50	TDF+3TC+EFV	Unknown	Positive, COI: 131	Positive VT: 19.85	gp36: – gp140: – p31: – gp160: ++ p24: – gp41: ++	2.93 log	HIV-1 positive
1	Indeterminate	Unknown	38.1	Unknown	Unknown	Unknown	Positive, COI: 118.7	Positive, VT: 17.28	gp36: –gp140: – p31: – gp160: + p24: + gp41: ++	2.75 log	HIV-1 positive
1	Indeterminate	Unknown	44.1	Unknown	Unknown	Unknown	Positive COI: 53.47	Positive VT: 16.85	gp36: – gp140: – p31: – gp160: + p24: – gp41: +	2.75 log	HIV-1 positive

ART, antiretroviral therapy; COI, cut-off index; DBS, dried blood spots; EFV, efavirenz; RDT, rapid diagnostic test; SD, standard deviation; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; VT, test value.
[a] Result interpretation of Geenius assay: no line (–), low intensity line (+), and high intensity line (++).

and the loss of credibility of the HIV testing program (Kufa et al., 2017). The misdiagnosis at some HIV testing sites in sub-Saharan Africa, and inconsistent findings regarding the accuracy of widely used simple diagnostic tests, have highlighted the urgent need for a comprehensive evaluation of these tests and the algorithm applied, with special emphasis on their performance by geographical location and other characteristics. The algorithm used in Kinshasa (DRC) includes the use of three RDTs for the diagnosis and confirmation of HIV. However, the RDTs are not the optimal option for confirmation of the diagnosis, although this algorithm is better than others used in many countries as the tie-breaker algorithm, which have been shown to have more false-positive results (Shanks et al., 2015).

Twenty-three DBS samples from the study population belonged to individuals who had received an indeterminate RDT-based HIV diagnosis in DRC. Among them, three patients were actually infected with HIV and had detectable RNA according to the DBS analysis, but only one (a 25-year-old female) was receiving ART with TDF/EFV/3TC. The remaining two patients showed a low level viremia and a reduced level of antibody based on the chemiluminescence signal and the Geenius intensity bands, probably related to an asymptomatic stage of HIV infection or clinical latency that could lead to indeterminate RDT results (Table 3). On the other hand, 11 samples (3.6%, 11/303; from nine different patients) initially classified as positive in the origin, resulted negative after DBS serological and molecular analysis. These nine cases of HIV misdiagnosis corresponded to three persons newly diagnosed during the study period (2016–2018) and six individuals who had received an RDT-based diagnosis years before, confirming the need for confirmatory testing prior to initiating ART, either by conducting a confirmatory test locally if available, or by studying samples such as DBS at an external laboratory where additional serological and/or molecular analysis can be performed. Up to three false-positive samples corresponded to a patient misdiagnosed in 2016 with a high CD4 level (925–1029 CD4/µl), whose ART was withdrawn after 2 years of unnecessary treatment. These findings could be explained either by poor performance of the RDT algorithm, or by poor performance of the DBS samples.

Rapid immunochromatographic tests for HIV are recommended in low resource settings due to poor infrastructure and geographic limitations, but the low HIV prevalence among the general population in DRC (0.7%) may be associated with a lower positive predictive value for these methods (Rubio-Garrido et al., 2019, UNAIDS. UNAIDS 2020, Lasry et al., 2019, Eaton et al., 2017, Dupwa et al., 2019, Tan et al., 2016). Misdiagnosis can also be influenced by subjective reading of tests, not respecting the incubation time, or incorrect labelling (Tuaillon et al., 2020). Even fourth-generation RDTs used in HIV diagnosis algorithms have led to a significant number of false-positive results delivered. Several authors have evaluated RDT-based diagnosis using the Determine HIV 1/2 (Abbott) assay and have detected a false positivity rate of up to 31.5% (Kosack et al., 2017, Kosack et al., 2017). Most HIV RDT kits available in SSA have been evaluated as individual assays and not as testing algorithms; therefore, each country must choose and standardize its own testing algorithm based on local costs, storage issues, human resources, available facilities and infrastructure, service demand, and local geography (Kaleebu et al., 2018, Plate and Wiktor, 2007).

On the other hand, DBS analysis cannot give an immediate result but can be performed with centralized laboratory technology. Both serological and molecular analysis can be implemented successively in different steps for HIV diagnosis and typing; even drug levels can be measured to assess patient adherence. A number of authors have reported adequate sensitivity and specificity for HIV diagnosis through DBS analysis (Rubio-Garrido et al., 2019, De Mulder and Holguín, 2013, Tuaillon et al., 2020, Fernández McPhee

et al., 2015, Stefic et al., 2020), and both chronic and acute infections can be diagnosed reliably (Dupwa et al., 2019). The study data showed high sensitivity and specificity for both chemiluminescent assays used with DBS samples (Elecsys and VIDAS), similar to the result reported recently by Stefic et al. (98.8%) (Stefic et al., 2020).

The implementation of DBS for viral load determination is considered one of the most necessary immediate measures to reduce the HIV-related mortality rate in Africa (Phillips et al., 2016). In the present study, all of the samples were analysed by COBAS AmpliPrep/COBAS Taqman HIV-1 (Roche) to confirm the diagnosis and monitor infection and transmissibility (Powers et al., 2011). This viral load platform has been reported to be a reliable method to detect and quantify the wide variety of subtypes and recombinants currently circulating in DRC from DBS samples (Rubio-Garrido et al., 2019).

Based on the study results, the Geenius HIV 1/2 confirmatory assay can be a reliable tool to avoid misdiagnosis. It offered excellent specificity for HIV diagnosis working with DBS samples. However, the sensitivity of the Geenius assay on DBS was 91.7% in this series, since up to nine samples from true HIV-1 patients showed a negative result by Geenius. Therefore, this test may be very suitable as a confirmatory test, but not as a screening test. Other authors have reported that it exhibits better sensitivity and better performance in detecting HIV-1 infections than other confirmatory immunoassays (Serhir et al., 2019, Hallen et al., 2014, Mor et al., 2014). In the current series, no HIV-2 infection was detected among the participants. Studies have mainly described HIV-2 infections in West African countries (Visseaux et al., 2016).

This study has a number of limitations. First, DBS were not compared to plasma due to the lack of paired plasma/DBS specimens collected from each subject in the study population. Second, only 135 out of the 365 samples could be tested with the Geenius HIV 1/2 assay. However, this random selection coupled with RNA detection from all DBS samples allowed discrepant results to be resolved and acute infections to be ruled out. Regarding the viral load and serology evaluation, among adults diagnosed as HIV-positive or indeterminate by RDTs, 36% had received ART before sampling and their RDT diagnosis had been conducted prior to DBS collection for this study. Finally, storage conditions could influence the DBS analysis; however, specimens were transported on dry ice and preserved at −80°C until use.

In conclusion, the main strength of this study is showing the usefulness of DBS testing to confirm or rule out HIV infection in low-income countries. Assessing HIV misdiagnosis in SSA is a public health priority, as an incorrect diagnosis can lead to unnecessary treatment and spreads discriminatory attitudes and stigma in these regions. The results of this study may have a direct clinical impact on the global diagnosis and follow-up of HIV infection, since the use of RDT-based HIV diagnostic algorithms with suboptimal reliability, in areas with very limited resources, may further delay the achievement of the 95–95–95 objectives proposed for 2030.

Funding

This study was funded by the Government of Spain (Fondo de Investigación en Salud-FI, grants PI16/01908) and the Government of Navarre (grant 045-2015).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the patients who participated in the study, as well as the local professionals responsible for the DBS and data collection from enrolled patients at Monkole and Kalembelembe hospitals in Kinshasa. We thank Patricia Sanz for her technical support during laboratory processing of DBS samples. We are grateful for the support of the Institute for Tropical Health (Obra Social la CAIXA and Fundación Caja Navarra, Fundación María Francisca de Roviralta, Ubesol and Inversiones Garcilaso de la Vega S.L.).

References

Gisslén M, Svedhem V, Lindborg L, Flamholc L, Norrgren H, Wendahl S, et al. Sweden, the first country to achieve the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) 90-90-90 continuum of HIV care targets. HIV Med 2017;18(4):305–7.

Guichet E, Aghokeng A, Serrano L, Bado G, Toure-Kane C, Eymard-Duvernay S, et al. Short Communication: High Viral Load and Multidrug Resistance Due to Late Switch to Second-Line Regimens Could Be a Major Obstacle to Reach the 90-90-90 UNAIDS Objectives in Sub-Saharan Africa. AIDS Res Hum Retroviruses 2016;32(12):1159–62.

Hemelaar J, Elangovan R, Yun J, Dickson-Tetteh L, Fleminger I, Kirtley S, et al. Global and regional molecular epidemiology of HIV-1, 1990–2015: a systematic review, global survey, and trend analysis. Lancet Infect Dis 2019;19(2):143–55.

Rubio-Garrido M, González-Alba JM, Reina G, Ndarabu A, Barquín D, Carlos S, et al. Current and historic HIV-1 molecular epidemiology in paediatric and adult population from Kinshasa in the Democratic Republic of Congo. Sci Rep. 2020;10(1):1–13.

Rubio-Garrido M, Ndarabu A, Reina G, Barquín D, Fernández-Alonso M, Carlos S, et al. Utility Of POC Xpert HIV-1 Tests For Detection-Quantification Of Complex HIV Recombinants Using Dried Blood Spots From Kinshasa. D. R. Congo. Sci Rep. 2019;9(1):1–11.

De Mulder M, Holguín Á. Dried blood spots for monitoring HIV infection in Public Health Programs in developing countries. Enferm Infecc Microbiol Clin 2013;31(2):100–7.

UNAIDS. UNAIDS. Joint United Nations Programme on HIV/AIDS. UNAIDS data 2020. Geneva, Switzerland: UNAIDS; 2020.

Vidal N, Mulanga-Kabeya C, Nzilambi N, Delaporte E, Peeters M. Identification of a complex env subtype E HIV type 1 virus from the Democratic Republic of Congo, recombinant with A, G, H, J, K, and unknown subtypes. AIDS Res Hum Retroviruses 2000;16(18):2059–64.

Shanks L, Klarkowski D, O'Brien DP. False Positive HIV Diagnoses in Resource Limited Settings: Operational Lessons Learned for HIV Programmes. PLoS One 2013;8(3):8–13.

Kufa T, Kharsany AB, Cawood C, Khanyile D, Lewis L, Grobler A, et al. Misdiagnosis of HIV infection during a South African community-based survey: Implications for rapid HIV testing. J Int AIDS Soc 2017;20(6):35–43 Suppl.

; 2019. p. 292 p.

Do Rosario Augusto A, Iriemenam NC, Kohatsu L, De Sousa L, Maueia C, Hara C, et al. High level of HIV false positives using EIAbased algorithm in survey: Importance of confirmatory testing. PLoS One 2020;15:1–12 10 October.

Kosack CS, Shanks L, Beelaert G, Benson T, Savane A, Ng'Ang'A A, et al. HIV misdiagnosis in sub-Saharan Africa: Performance of diagnostic algorithms at six testing sites: Performance. J Int AIDS Soc 2017;20(1):1–18.

Klarkowski D, O'Brien DP, Shanks L, Singh KP. Causes of false-positive HIV rapid diagnostic test results. Expert Rev Anti Infect Ther 2014;12(1):49–62.

Tuaillon E, Kania D, Pisoni A, Bollore K, Taieb F. Ontsira Ngoyi EN, et al. Dried Blood Spot Tests for the Diagnosis and Therapeutic Monitoring of HIV and Viral Hepatitis B and C. Front Microbiol 2020;11(March):1–7.

Fernández McPhee C, Álvarez P, Prieto L, Obiang J, Avedillo P, Vargas A, et al. HIV-1 infection using dried blood spots can be confirmed by Bio-Rad Geenius™ HIV 1/2 confirmatory assay. J Clin Virol 2015;63:66–9.

Miri-Dashe T, Osawe S, Daniel MTN, Choji RP, Mamman I, Deme K, et al. Comprehensive reference ranges for hematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. PLoS One 2014;9(5).

Lasry A, Kalou MB, Young PR, Rurangirwa J, Parekh B. Behel S. Cost implications of HIV retesting for verification in Africa. PLoS One 2019;14(7).

Eaton JW, Johnson CC, Gregson S. The Cost of Not Retesting: Human Immunodeficiency Virus Misdiagnosis in the Antiretroviral Therapy "Test-and-Offer" Era. Clin Infect Dis 2017;65(3):522–5.

WHO. Implementing HIV Viral Load Testing. 2014;(July).

Stefic K, Guinard J, Peytavin G, Saboni L, Sommen C, Sauvage C, et al. Screening for human immunodeficiency virus infection by use of a fourth-generation antigen/antibody assay and dried blood spots: In-depth analysis of sensitivity and performance assessment in a cross-sectional study. J Clin Microbiol 2020;58(1):1–10.

Dupwa B, Kumar AMV, Tripathy JP, Mugurungi O, Takarinda KC, Dzangare J, et al. Retesting for verification of HIV diagnosis before antiretroviral therapy initiation in Harare, Zimbabwe: Is there a gap between policy and practice? Trans R Soc Trop Med Hyg 2019;113(10):610–16.

Kosack S, CaraPage A-L, Savane A, Ng'ang'a A, Zahinda J-PB, Shanks L, Fransen K, et al. Towards more accurate HIV testing in sub-Saharan Africa: a multi-site

evaluation of HIV RDTs and risk factors for false positives. J Int AIDS Soc 2017;20(1):22098.
Shanks L, Siddiqui MR, Kliescikova J, Pearce N, Ariti C, Muluneh L, et al. Evaluation of HIV testing algorithms in Ethiopia: The role of the tie-breaker algorithm and weakly reacting test lines in contributing to a high rate of false positive HIV diagnoses. BMC Infect Dis 2015;15(1):1–10.
Tan WS, Chow EPF, Fairley CK, Chen MY, Bradshaw CS, Read TRH. Sensitivity of HIV rapid tests compared with fourth-generation enzyme immunoassays or HIV RNA tests. Aids 2016;30(12):1951–60.
Kosack CS, Shanks L, Beelaert G, Benson T, Savane A, Ng'ang'a A, et al. Designing HIV testing algorithms based on 2015 WHO guidelines using data from six sites in sub-Saharan Africa. J Clin Microbiol 2017;55(10):3006–15.
Kaleebu P, Kitandwe PK, Lutalo T, Kigozi A, Watera C, Nanteza MB, et al. Evaluation of HIV-1 rapid tests and identification of alternative testing algorithms for use in Uganda. BMC Infect Dis 2018;18(1):1–14.
Plate DK, Wiktor SZ. Evaluation and implementation of rapid HIV tests: The experience in 11 African countries. AIDS Res Hum Retroviruses 2007;23(12):1491–8.
Phillips A, Vojnov L, Cohn J, Roberts T, Ellman T, Bonner K, et al. Sustainable HIV Treatment in Africa through Viral Load-Informed Differentiated Care. Nature 2016;528(7580):S68–76.
Powers KA, Ghani AC, Miller WC, Hoffman IF, Pettifor AE, Kamanga G, et al. The Role of Acute and Early HIV Infection in the Spread of HIV-1 in Lilongwe, Malawi: Implications for "Test and Treat" and Other Transmission Prevention Strategies. Lancet 2011;378(9787):256–68.
Serhir B, Desjardins C, Doualla-Bell F, Simard M, Tremblay C, Longtin J. Evaluation of the bio-rad geenius HIV 1/2 assay as part of a confirmatory HIV testing strategy for Quebec, Canada: Comparison with western blot and inno-lia assays. J Clin Microbiol 2019;57(6):1–9.
Hallen AH, Samuelson A, Nordin M, Albert J, Bogdanovic G. Evaluation of bio-rad geenius HIV-1 and -2 assay as a confirmatory assay for detection of HIV-1 and -2 antibodies. Clin Vaccine Immunol 2014;21(8):1192–4.
Mor O, Mileguir F, Michaeli M, Levy I, Mendelson E. Evaluation of the bio-rad geenius HIV 1/2 assay as an alternative to the INNO-LIA HIV 1/2 assay for confirmation of HIV infection. J Clin Microbiol 2014;52(7):2677–9.
Visseaux B, Damond F, Matheron S, Descamps D, Charpentier C. Hiv-2 molecular epidemiology. Infect Genet Evol 2016;46:233–40.