Journal of Clinical Virology 62 (2015) 92–97

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Performances of fourth generation HIV antigen/antibody assays on filter paper for detection of early HIV infections

Dramane Kania[a,b,*,1], Tam Nguyen Truong[a,1], Ana Montoya[a], Nicolas Nagot[a,c], Philippe Van de Perre[a,d], Edouard Tuaillon[a,d]

[a] Université Montpellier 1, INSERM U 1058, 34394 Montpellier, France
[b] Laboratoire de Virologie, Unité VIH et Maladies Associées, Centre Muraz, Bobo-Dioulasso, Burkina Faso
[c] CHU Montpellier, Département d'Information Médicale, 34295 Montpellier, France
[d] CHU Montpellier, Département de Bactériologie-Virologie, 34295 Montpellier, France

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form 31 October 2014
Accepted 3 November 2014

Keywords:
HIV-1
Acute infection
Point-of-care
Dried blood spot
4th generation immunoassay
p24 antigen

ABSTRACT

Background: Point-of-care testing and diagnosis of HIV acute infections play important roles in preventing transmission, but HIV rapid diagnosis tests have poor capacity to detect early infections. Filter paper can be used for capillary blood collection and HIV testing using 4th generation immunoassays.
Objectives: Antigen/antibody combined immunoassays were evaluated for their capacity to identify early HIV infections using filter paper in comparison with rapid test.
Study design: Thirty nine serum samples collected from HIV seroconverters were spotted onto filter paper and tested by the Roche Elecsys® HIV Combi PT test and the DiaSorin Liaison XL Murex HIV Ab/Ag assay.
Results: Fourth generation immunoassays identified 34 out of 39 HIV early infections using dried serum spot, whereas the Determine™ HIV-1/2 rapid test detected 24 out of 39 HIV positive serum (87.2% vs 61.5% respectively, $p = 0.009$). p24 antigen was detected by the Liaison XL in 19 dried serum samples (48.7%). In the group characterized by a negative western blot, 7 out of 8 (87.5%) and 6 out of 8 (75.0%) samples were found positive for HIV using the Elecsys and the Liaison XL, respectively. None of these eight samples classified in this group of early acute infections were found positive by the rapid test.
Conclusion: Fourth generation Ag/Ab immunoassays performed on dried serum spot had good performance for HIV testing during the early phases of HIV infection. This method may be useful to detect HIV early infections in hard-to-reach populations and individuals living in remote areas before rapid tests become positive.

1. Background

Acute infection plays a significant role in transmission of human immunodeficiency virus (HIV) because of high viral loads encountered during this phase of infection [1]. It is of particular importance to identify persons with acute infections to prevent transmission and to begin treatment, but this diagnosis remains a challenge. Anti-HIV-1 antibodies can be detected in blood about 3–4 weeks after infection in most individuals. The window period for HIV diagnosis can be shortened by several days by testing plasma HIV-1 RNA and p24 antigen.

The HIV RNA detection is superior to p24 antigen assays in detecting viral particles. It has been estimated that RT-PCR methods detect HIV-1 infection three to five days earlier than the p24 antigen test and one to three weeks earlier than standard serologic tests [1]. However, the screening for HIV infection remains based on serological testing alone because of the cost and complex technology of molecular assays [2].

Fourth generation immunoassays (IAs) have been developed in the last 10 years to improve HIV diagnosis especially during early phases of the infection by coupling HIV-1/HIV-2 antigen sandwich assays that detect both IgG and IgM class antibodies (3rd generation test) with the detection of p24 antigen [3–8]. However, these tests required venipuncture and laboratory equipments which limit their use.

Point-of-care testing based on HIV rapid diagnostic tests (RDTs) have been recommended due to their reliability, simplicity to use and accessibility for people living in remote areas [9]. Blood collection on filter paper represents also an interesting device to

* Corresponding author at: Laboratoire de Virologie, Unité VIH et Maladies Associées, Centre Muraz, BP390 Bobo-Dioulasso 01, Burkina Faso. Tel.: +226 20 97 01 02; Fax: +226 20 97 04 57.
E-mail address: kaniadramane@yahoo.fr (D. Kania).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jcv.2014.11.005
1386-6532/

Table 1
Patient characteristics.

	WB negative (Group 1, *n* = 8)	WB indeterminate (Group 2, *n* = 23)	WB positive (Group 3, *n* = 8)	Total *N* = 39
Age (year) ± SD	43.5 ± 15.3	38.9 ± 10.7	37.6 ± 8.7	39.6 ± 11.3
Male, n (%)	7 (87.5%)	21 (91.3%)	7 (87.5%)	35 (89.7%)
Risk factor, n (%)				
MSM	5 (62.5%)	9 (39.1%)	3 (37.5%)	17 (43.6%)
Heterosexuality	2 (25.0%)	3 (13.0%)	2 (25.0%)	7 (17.9%)
IDU	0 (0.0)	1 (4.4%)	0 (0.0)	1 (2.6%)
Unknown	1 (12.5%)	10 (43.5%)	3 (37.5%)	14 (35.9%)
CD4 mean count ± SD	317.3 ± 165.7	430.6 ± 221.2	436.9 ± 190.5	409.8 ± 206.0
CD4 median count (IQR 25–75)	273.0 (248.5–368.0)	375.5 (283.0–588.5)	473.0 (345.0–570.0)	355.0 (269.5–556.0)
CD8 mean count ± SD	354.6 ± 203.3	917.0 ± 591.8	940.7 ± 430.3	812.3 ± 548.7
CD8 median count (IQR 25–75)	286.0 (185.0–548.5)	865.0 (471.8–1,191.0)	902.0 (683.5–1,024.5)	683.5 (425.8–1,022.0)
HIV RNA mean concentration (Log_{10} copies/mL) ± SD	6.6 ± 0.7	6.0 ± 0.9	5.5 ± 1.1	6.6 ± 6.6
HIV RNA median concentration (IQR 25–75) (Log_{10} copies/mL)	7.0 (6.5–7.0)	6.0 (5.5–6.8)	5.9 (5.0–6.1)	6.1 (5.5–6.8)
HIV genotype	B (2); CRF02_AG (5); CRF31_BC (1)	B (19); CRF02_AG (1); NA (3)	B (5); F1 (1); NA (2)	B (26); CRF02_AG (6); CRF31_BC (1); F1 (1); NA (5)
HIV p24 reactive, n (%)	8 (100.0%)	23 (100.0%)	7 (87.5%)	38 (97.4%)
HIV p24 mean concentration (pg/mL) ± SD	11,878.6 ± 19,218.7	3,263.3 ± 10,619.0	276.7 ± 599.8	4,843.4 ± 12,691.9
HIV p24 median concentration (IQR 25–75) (pg/mL)	421.0 (359.5–500.0)	145.5 (29.4–383.0)	36.2 (12.5–122.3)	169.0 (36.6–410.5)

WB, western blot; *n*, number; MSM, men who have sex with men; IDU, injection drug user; HIV, human immunodeficiency virus; RNA, ribonucleic acid; SD, standard deviation; IQR, inter-quartile range; mL, milliliter; pg, picogram; log_{10}, logarithm of 10; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; NA, not available; CRF, circulating recombinant form.

improved diagnosis of infectious diseases in hard-to-reach populations. Blood samples collected on filter paper at the site of patient care can be tested in laboratory by means of 4th generation ELISA as a reliable alternative or a complementary approach for HIV diagnosis in resource-limited settings [10]. Many studies have demonstrated that dried blood spot (DBS) and dried serum spot (DSS) are useful for HIV diagnosis and monitoring with an excellent analytical performance [11]. However no data are available for these sample collection devices, and their transportation and storage for the diagnosis of acute HIV infections.

2. Objectives

This study was conducted to evaluate the performance of HIV 4th generation immunoassays to detect acute HIV infections using DSS in comparison to serum specimen and rapid diagnostic test.

3. Study design

3.1. Population, specimens and data collection

A total of 39 serum samples from newly diagnosed HIV infected persons collected at early phases of infection and archived in the Virology Laboratory of Montpellier University Hospital were selected. HIV early infections were established based on p24 antigen/anti-HIV antibodies fourth generation EIA, plasma HIV RNA quantification and immunoblotting assay (WB), and clinical manifestations. Patient characteristics are presented in Table 1.

A positive WB result is defined as the presence of at least two clearly visible *Env* glycoprotein bands and at least one clear line of *Gag* or *Pol* protein. An indeterminate WB result is the presence of any other band or bands that fail to meet the criteria for positivity. A negative WB result is the absence of all bands.

The p24-antigen has been performed by the Vidas® HIV p24 II assay (BioMérieux SA, Marcy-l'Etoile, France). RNA viral load has been performed using Roche Cobas system (dynamic range of 20–10,000,000 copies/mL) with the Cobas AmpliPrep/Cobas Amplicor HIV-1 Monitor Test, v1.5 or the Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, v2.0 (Roche Diagnostics GmbH, Mannheim, Germany).

3.2. HIV rapid testing in the study

Serum samples were assessed by means of an immunochromatographic rapid diagnostic tests (RDTs) using Alere Determine™ HIV-1/2 assay (Alere Medical Co. Ltd., Chiba, Japan). Fifty microliters (50 μL) of serum of each patient were directly performed as recommended by the manufacturer instructions and results obtained after 15 min.

3.3. HIV acute infection screening using p24 antigen/antibody combination assays on filter paper

From each selected serum sample, about 50 μL of serum were spotted onto Whatman 903™ Protein Saver Card (Whatman GmbH, Dassel, Germany) and dried at ambient temperature for at least 3 h. Filter paper cards were then stocked at −20 °C before use. Samples spotted on filter paper card were tested using two 4th generation immunoassays (IAs): (i) Elecsys® HIV Combi PT assay (Roche Diagnostics GmbH, Mannheim, Germany) on an automated system (Cobas 6000/Cobas e601, Roche Diagnostics GmbH); and (ii) Liaison® XL Murex HIV Ab/Ag test (DiaSorin S.p.A., Saluggia, Italy) on a fully automated chemiluminescence analyzer (Liaison® XL, DiaSorin S.p.A.). This test distinguishes between signals from antibody and antigen reactivities [3]. Assays were performed and results interpreted in accordance with the manufacturer's instructions.

3.4. Filter paper performing by means of Elecsys® HIV Combi PT assay

Six mm diameter of disk punched from each dried serum spot (DSS) was eluted during 1 h into 200 μL of phosphate buffered saline (PBS) buffer with shaking. The final solution obtained from