HIV-1 can be Confirmed by Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay Using Dried Blood Spots

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Carolina Fernández McPhee[1], Patricia Alvarez[1], Luis Prieto[2], Jacinta Obiang[3], Pedro Avedillo[5], Antonio Vargas[5], Pablo Rojo[4], Jose Tomas Ramos[2] and Africa Holguín[1]

[1]Hospital Ramón y Cajal, IRyCIS, Microbiology Department-CIBERESP, Madrid, Spain, [2]Hospital de Getafe, Madrid, Spain, [3]Hospital Provincial de Bata, Ministerio de Sanidad y Bienestar Social, Equatorial Guinea, [4]Hospital Doce de Octubre, Madrid, Spain, [5]Centro Nacional de Medicina Tropical, Instituto de Salud Carlos III-Madrid, RICET, Spain

LABORATORIO de EPIDEMIOLOGÍA MOLECULAR del VIH-1
IRYCIS-Hospital Ramón y Cajal
Madrid, España

Correspondance:
1. **África Holguín:**
africa.holguin@salud.madrid.org
2. **Carolina Fernández McPhee**
carolina.fernandezmcphee@gmail.com
HIV-1 Molecular Epidemiology Laboratory
Microbiology Department, Hospital Ramón y Cajal
Ctra de Colmenar Viejo Km 9.100
28034, Madrid, Spain

ABSTRACT

Background: Confirmatory assays for HIV diagnosis are not well implemented in limited income settings, impeding an early HIV detection and, consequently, delaying the prescription of antiretroviral treatment in the infected population. Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay is a single-use immunochromatographic test for the confirmation and differentiation of individual antibodies of HIV-1 and HIV-2 in venous whole blood, serum or plasma samples. Since plasma/serum is difficult to collect and/or process in remote settings from limited resource countries and in mobile populations, our objective was to evaluate for the first time the assay Geenius™ HIV 1/2 for diagnostic use utilising dried blood spots (DBS) specimens.

Methodology: Geenius™ HIV 1/2 test was performed to confirm the HIV infection in 70 women previously diagnosed as HIV-1 positive by one to three rapid tests using whole blood samples in the Hospital of Bata, Equatorial Guinea, from November 2012 to August 2013. DBS specimens were prepared by adding two drops of blood to fill each circle of 903 filter paper cards (GE Healthcare). Cards were dried overnight at room temperature and stored at -20ºC until their shipping at room temperature to Madrid, Spain for testing. After their arrival, they were stored at -80ºC until use. Blood from one DBS circle per patient was eluted in 150 µl elution buffer for one hour with gentle rotation. The test was performed according to the manufacturer indications, but using 40 µl of the eluted circle as specimen. The results obtained from Geenius™ HIV 1/2 were confirmed by western blot (New Lav Blot I, Bio-Rad) using the same elution volume.

Results: Geenius™ HIV 1/2 (Bio-Rad) successfully confirmed the HIV-1 infection using DBS in all 70 tested women from Equatorial Guinea with at least one positive rapid test and only using a low volume of eluted dried blood. No HIV-1/HIV-2 coinfections were found in the study cohort. The HIV-1 positivity was confirmed in all cases in DBS by western blot (Bio-Rad).

Conclusions: This is the first report that proves a good performance of Geenius™ HIV 1/2 assay (Bio-Rad) for the confirmation of the HIV-1 infection using only two drops of dried blood. Our results aprove the utility of this rapid confirmatory assay using a low volumen of dried blood when a lack adequate infrastructure to collect, store or transport plasma or serum is found. DBS are a practical alternative to plasma for HIV serological diagnosis.

INTRODUCTION

- The diagnosis of the HIV infection in adults and children over 18 months of age is usually made using a serological screening test based on the detection of HIV 1/2 antibodies or the simultaneous detection of HIV 1/2 antibodies and HIV-1 p24 antigen. Enzyme immunoassays and rapid diagnostics tests (RDTs) can serve as first line assays. Reactive samples always need to be confirmed by serological confirmatory tests such as western blot.
- Due to their simplicity, cost and rapid turn-around time, WHO recommends the use of RDTs for the HIV detection in resource limited settings. Different RDTs are commercially available, including immunochromatographic (lateral-flow tests) and immunofiltration (flow-through tests) formats that use different specimens depending on the assay: fingerstick/capillary whole blood, venous whole blood, serum, plasma or oral fluid.
- In Equatorial Guinea, due to a HIV prevalence over 5% (6.2% in the population aged 15-49 years old), WHO recommends the confirmation of the HIV infection in reactive samples using a different second test and the same specimen if it is plasma or serum. If the specimen is fingerstick whole blood or dried blood on filter paper, WHO recommends the collection of a new sample.
- Dried blood spots (DBS) are an appropiate alternative for the diagnosis and confirmation of the HIV infection in areas where the population has limited access to health systems.
- Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay is a single-use immunochromatographic test for the simultaneous confirmation and differentiation of individual HIV 1/2 antibodies validated in venous whole blood, serum and plasma samples.

OBJECTIVE

To evaluate, for the first time, the HIV-1 and HIV-2 diagnostic performance of Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay using dried blood specimens from 70 HIV-infected women from Equatorial Guinea previously diagnosed as HIV positive using one to three rapid tests.

PRINCIPLE OF THE TEST

Figure 1: Geenius™ HIV 1/2 Confirmatory Assay (Bio-Rad). Test cassette



Test line 1: gp36 (HIV-2 envelope peptide)
Test line 2: gp140 (HIV-2 envelope peptide)
Test line 3: p31 (HIV-1 polymesare peptide)
Test line 4: gp160 (HIV-1 full length envelope recombinant protein)
Test line 5: p24 (HIV-1 full length core recombinant protein)
Test line 6: gp41 (Group M & O HIV-1 envelope peptides)
Test line 7: Control band (Protein A)

- This assay employs antigens for HIV-1 (p31, gp160, p24 and gp41) and for HIV-2 (gp36 and gp140), which are bound to the membrane solid phase and to the antibody binding Protein A. This Protein A is conjugated to colloidal dye gold particles.
- The sample is applied to the **sample+buffer well 1** with a waiting time of 5 minutes during which the sample and buffer migrate to the test strip. Additional buffer is added to the **buffer well 2** (15 minutes).
- If the sample contains HIV antibodies, they will be captured by the immobilized antigens in the test area (test lines 1-6) and the colloidal gold Protein A will bind to the captured antibodies producing pink/purple lines. The test control (test line 7) contains Protein A to ensure that the assay has worked properly.

Table 1: Interpretation of results

Positivity Criteria	Geenius HIV 1/2 Confirmatory Assay
Positive HIV-1	1 ENV HIV-1 + 1 GAG or 1 POL 2 ENV HIV-1
Positive HIV-2	2 ENV HIV-2
Negative	No band

METHODOLOGY

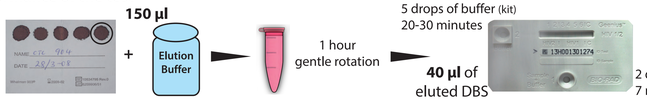
Sample panel

- 70 HIV-infected Equatorial Guinean women diagnosed as HIV-1 positive by on to three rapid tests in the Health Centre of María Rafols and in the Regional Hospital reference Centre Dr. Damián RokuEpitieMonanga (Equatorial Guinea) from November 2012 to August 2013.
- All individuals signed the informed consent and the local ethics committee approved the study protocol.

Specimen collection

- DBS were prepared in Equatorial Guinea by adding two drops of fingerstick whole blood on one circle of filter paper (Whatman no. 903).
- DBS were dried overnight at room temperature, placed in individual zip-lock bags containing silica desiccant and stored at -20ºC until their shipping to Madrid, Spain.
- Once in the laboratory in Madrid, DBS were stored at -80ºC until tested.

Performance of the test



RESULTS

- This is the first study using the Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay for the simultaneous detection of HIV 1/2 antibodies using DBS.
- HIV-1 antibodies were detected in all 70 DBS specimens from infected women in Equatorial Guinea and the HIV-1 infection was confirmed by western blot in all cases.
- No HIV-1/HIV-2 coinfections were found in the study cohort.
- Antibodies reactive to 4 or 3 HIV-1 antigens were found in 32 (45.7%) specimens and to 2 HIV-1 antigens in 6 (8.6%) samples.
- Most (95.7%) specimens presented reactivity to 2 Env proteins, 92.8% to Gag and 48.6% to Pol.

Table 2: Percentage of test bands and band profiles in the sample panel

HIV-1 bands			Number of patients per profile (%)
ENV	GAG	POL	
2	1	1	32 (45.7%)
2	1	0	30 (42.8%)
2	0	1	2 (2.9%)
2	0	0	3 (4.3%)
1	1	0	3 (4.3%)

Percentage of patients with each band			
2 ENV: 95.7% 1 ENV: 4.3%	1 GAG: 92.8% 0 GAG: 7.2%	1 POL: 48.6% 0 POL: 51.4%	70 (100%)

There was no reactivity to any of the HIV-2 test bands

CONCLUSIONS

- Geenius™ HIV 1/2 Confirmatory Assay can be used for the screening and confirmation of HIV-1 and/or HIV-2 using two drops of dried blood. It requires minimum laboratory infraestructure, following a simple and rapid performance procedure and a clear result interpretation.
- The use of DBS is a practical alternative to plasma or venous whole blood collected by venopunction in limited resource settings for the HIV serological diagnosis in adults and children over 18 months of age.

ACKNOWLEDGEMENTS

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