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Authors

Piwowar-Manning, Estelle
Fogel, Jessica M
Richardson, Paul
et al.

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Performance of the fourth-generation Bio-Rad GS HIV Combo Ag/Ab enzyme immunoassay for diagnosis of HIV infection in Southern Africa

Estelle Piwovar-Manning^a, Jessica M. Fogel^a, Paul Richardson^a, Shauna Wolf^a, William Clarke^a, Mark A. Marzinke^a, Agnès Fiamma^b, Deborah Donnell^{c,d}, Michal Kulich^e, Jessie K.K. Mbwambo^f, Linda Richter^{g,h}, Glenda Gray^{i,j}, Michael Sweat^k, Thomas J. Coates^l, and Susan H. Eshleman^{a,*}

Estelle Piwovar-Manning: epiwowa@jhmi.edu; Jessica M. Fogel: jfogel@jhmi.edu; Paul Richardson: pricha18@jhmi.edu; Shauna Wolf: swolf14@jhmi.edu; William Clarke: wclarke@jhmi.edu; Mark A. Marzinke: Mmarzin1@jhmi.edu; Agnès Fiamma: afiamma@gmail.com; Deborah Donnell: Deborah@sharp.org; Michal Kulich: kulich@karlin.mff.cuni.cz; Jessie K.K. Mbwambo: jmbwambo@gmail.com; Linda Richter: lrichter@hsr.ac.za; Glenda Gray: Glenda.Gray@mrc.ac.za; Michael Sweat: sweatm@musc.edu; Thomas J. Coates: coates@mednet.ucla.edu; Susan H. Eshleman: seshlem@jhmi.edu

^aDepartment of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

^bProgram in Global Health, University of California at Los Angeles, Los Angeles, CA, USA

^cVaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

^dDepartment of Global Health, University of Washington, Seattle, WA, USA

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*Corresponding author at: Department of Pathology, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Room 646 Ross Building, Baltimore, MD 21205, USA. Tel.: +1 410 614 4734; fax: +1 410 502 9244.

Competing interests

None of the authors has a conflict of interest or potential conflict of interest, with the following exception: Dr. Eshleman has collaborated on research studies with investigators from Abbott Laboratories. Abbott Laboratories has provided reagents and performed testing for some collaborative studies. Dr. Eshleman received an honorarium in 2009 for a presentation at a symposium sponsored by Abbott Laboratories.

Ethical approval

The work described was carried out in accordance with the Declaration of Helsinki. Samples were obtained from the NIMH Project Accept Study (HPTN 043). This study was done in close partnership with established community advisory boards and local government departments. Oral consent was obtained at the community level for trial participation and randomization. Participation in all intervention activities was voluntary. To approach household members to participate in the post-intervention assessment, investigators needed permission from the head of the household. Oral consent was obtained from each participant for each component of data collection and for collection and testing of blood samples. The study was approved by ethics committees for each site and by all participating academic institutions.

Authors contributions

All authors have made substantial contributions to the work. All authors contributed to preparation of the manuscript and approved the final version of the manuscript. Estelle Piwovar-Manning was involved in designed the study, coordinated testing and responsible for data analysis and interpretation. Jessica M. Fogel was involved in coordinated the project, analyzed test results and drafted the manuscript. Paul Richardson and Shauna Wolf were involved in coordinated and performed HIV testing and analyzed test results. William Clarke and Mark A. Marzinke were responsible for antiretroviral drug testing and interpretation of test results. Agnès Fiamma was a study coordinator for NIMH Project Accept (HPTN 043) and provided data for the study. Deborah Donnell and Michal Kulich were the statistician for NIMH Project Accept (HPTN 043) and provided data for the study. Jessie K.K. Mbwambo, Linda Richter and Glenda Gray were site PI for NIMH Project Accept (HPTN 043) and provided samples and data for the study. Michael Sweat was a investigator for NIMH Project Accept (HPTN 043) and provided samples and data for the study. Thomas J. Coates was a PI of NIMH Project Accept (HPTN 043) and provided samples and data for the study. Susan H. Eshleman was involved in designed the study, responsible for data analysis and interpretation, and drafted the manuscript.

^eDepartment of Probability and Statistics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic

^fMuhimbili University of Health and Allied Sciences, Muhimbili University Teaching Hospital, Dar es Salaam, Tanzania

^gDST-NRF Centre of Excellence in Human Development, Universities of the Witwatersrand and KwaZulu-Natal, South Africa

^hHuman Sciences Research Council, Durban, South Africa

ⁱPerinatal HIV Research Unit, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Johannesburg, South Africa

^jSouth African Medical Research Council, Cape Town, South Africa

^kDepartment of Psychiatry and Behavioral Sciences, The Medical University of South Carolina, Charleston, SC, USA

^lCenter for World Health, David Geffen School of Medicine and UCLA Health, Los Angeles, CA, USA

Abstract

Background—Fourth-generation HIV assays detect both antigen and antibody, facilitating detection of acute/early HIV infection. The Bio-Rad GS HIV Combo Ag/Ab assay (Bio-Rad Combo) is an enzyme immunoassay that simultaneously detects HIV p24 antigen and antibodies to HIV-1 and HIV-2 in serum or plasma.

Objective—To evaluate the performance of the Bio-Rad Combo assay for detection of HIV infection in adults from Southern Africa.

Study design—Samples were obtained from adults in Soweto and Vulindlela, South Africa and Dar es Salaam, Tanzania (300 HIV-positive samples; 300 HIV-negative samples; 12 samples from individuals previously classified as having acute/early HIV infection). The samples were tested with the Bio-Rad Combo assay. Additional testing was performed to characterize the 12 acute/early samples.

Results—All 300 HIV-positive samples were reactive using the Bio-Rad Combo assay; false positive test results were obtained for 10 (3.3%) of the HIV-negative samples (sensitivity: 100%, 95% confidence interval [CI]: 98.8–100%); specificity: 96.7%, 95% CI: 94.0–98.4%). The assay detected 10 of the 12 infections classified as acute/early. The two infections that were not detected had viral loads < 400 copies/mL; one of those samples contained antiretroviral drugs consistent with antiretroviral therapy.

Conclusions—The Bio-Rad Combo assay correctly classified the majority of study specimens. The specificity reported here may be higher than that seen in other settings, since HIV-negative samples were pre-screened using a different fourth-generation test. The assay also had high sensitivity for detection of acute/early infection. False-negative test results may be obtained in individuals who are virally suppressed.

Keywords

Fourth-generation; HIV; Diagnosis; Africa; Enzyme immunoassay

1. Background

The GS HIV Combo Ag/Ab EIA (Bio-Rad Combo, Bio-Rad Laboratories, Redmond, WA) is a fourth-generation antigen/antibody enzyme immunoassay (EIA) that is approved by the United States (US) Food and Drug Administration as an aid to diagnose human immunodeficiency virus (HIV) infection [1,2]. The assay detects HIV-1 p24 antigen, in addition to anti-HIV antibodies (IgG, IgM, and IgA; HIV-1 groups M and O and/or HIV-2) [3], which facilitates detection of acute and early HIV infection. The package insert reports a sensitivity of 100% (95% confidence interval [CI]: 99.70–100.0%) and a specificity of 99.87% (95% CI: 99.76–99.93%) in individuals at low risk of HIV infection [4]. In previous studies, the assay demonstrated high sensitivity [3] and high specificity in both low- and high-risk groups [3,5–7]. The assay detected 86–95% of acute HIV infections [3] and detected acute infection on average 8.8 days earlier than a third-generation HIV assay [8]. However, limited data are available for performance of the assay for HIV diagnosis in Southern Africa [3,8]. We evaluated the performance of the Bio-Rad Combo assay by testing plasma samples from South Africa and Tanzania. In South Africa, the overwhelming majority of infections are subtype C [9]. In Tanzania, HIV infections include varied subtypes (mostly A, C, and D), as well as intersubtype recombinant strains [9,10].

2. Objective

The aim of this study was to evaluate the performance of the Bio-Rad Combo assay for diagnosis of HIV infection in adults from Southern Africa.

3. Study design

3.1. Samples used for analysis

Plasma samples were collected at three study sites (Soweto and Vulindlela, South Africa; Kisarawe, Tanzania) in the National Institute of Mental Health (NIMH) Project Accept HIV Prevention Trials Network (HPTN) 043 study (NCT00203749) [10,11]. In a previous study, the subtype distribution in the HPTN 043 communities in Tanzania was estimated as: 44.3% subtype A, 22.1% subtype C, 9.7% subtype D, and 23.9% intersubtype recombinant [10]. Plasma samples were initially analyzed in laboratories in South Africa (plasma) and Tanzania (whole blood) using two HIV rapid tests performed in parallel: the Determine HIV-1/2 assay (Inverness Medical Innovations/Alere, Phetchabun, Japan) and the SD Bioline HIV 1/2 v3 assay (Standard Diagnostics, Youngin-Si, South Korea). The Uni-Gold HIV assay (Trinity Biotech, Bray, Ireland) was also used in Soweto as an alternate assay for a subset of samples. Samples were provisionally classified as HIV POS (two reactive rapid tests) or HIV NEG (two non-reactive rapid tests) [12]; samples with discordant HIV rapid tests were not evaluated in this study. The samples were further characterized in a previous study to determine the primary endpoint of the HPTN 043 study (HIV incidence) [10]. Testing algorithms used to identify acute/early HIV infections in the HPTN 043 cohort are

described in a previous report [10]. Briefly, an infection was classified as acute if the sample was HIV RNA positive and HIV antibody negative and early if the sample was HIV RNA positive, EIA positive, and Western blot indeterminate [10].

3.2. Evaluation of the Bio-Rad Combo assay

A randomly-selected subset of samples from HPTN 043 was tested with the Bio-Rad Combo assay. This included 300 HIV-positive samples (100 from each study site) and 300 HIV-negative samples (100 from each study site) (Fig. 1). The 300 HIV-negative samples selected for testing were non-reactive using the fourth-generation ARCHITECT HIV Ag/Ab Combo assay (Abbott Combo, Abbott Laboratories, Wiesbaden, Germany) (Fig. 1). HIV-negative samples that were reactive using the Bio-Rad Combo assay were also tested using the APTIMA HIV-1 RNA Qualitative Assay (Aptima RNA; Hologic Gen-Probe Inc., San Diego, CA) and the Multispot HIV-1/HIV-2 Rapid Test (Multispot; Bio-Rad Laboratories, Redmond, WA); both HIV-1 indicators in the Multispot assay were required to be present to interpret the test result as positive.

Samples from individuals previously classified as having acute/early infection were tested with the Bio-Rad Combo assay and a panel of additional assays that included the third-generation VITROS Anti-HIV 1 + 2 assay (Ortho Clinical Diagnostics, Rochester, NY), the fourth-generation Abbott Combo assay, the Genetics System HIV-1 Western blot test (Bio-Rad Laboratories, Redmond, WA, USA), the Aptima RNA assay, and a viral load assay (Roche COBAS AMPLICOR HIV-1 MONITOR test, v1.5, Roche Diagnostics, Branchburg, NJ). Samples with viral loads <400 copies/mL were tested using a qualitative assay that detects 15 antiretroviral (ARV) drugs (nine protease inhibitors, four nucleoside/nucleotide reverse transcriptase inhibitors, and two non-nucleoside reverse transcriptase inhibitors) [13].

4. Results

All 300 of the HIV-positive samples were reactive using the Bio-Rad Combo assay (Fig. 1A); 290 (96.7%) of the 300 HIV-negative samples were non-reactive using this assay (Fig. 1B). In this African cohort, the sensitivity of the Bio-Rad Combo assay was 100% (95% confidence interval [CI]: 98.8–100%) and the specificity was 96.7% (95% CI: 94.0–98.4%). The ten HIV-negative samples that were reactive with the Bio-Rad Combo assay were also tested using the Aptima RNA assay and the Multispot assay (see Methods). Nine of the 10 samples were non-reactive with both assays, indicating that the reactive results obtained with the Bio-Rad Combo assay were false-positive results. One sample was reactive for HIV-1 with the Multispot assay, but was non-reactive with the Aptima RNA assay. Since this sample was reactive with one fourth-generation assay (the Bio-Rad Combo assay) and the Multispot assay, it meets the criteria for HIV diagnosis using an HIV testing algorithm recently recommended for use in the US [14,15]. Because this sample was non-reactive using four other assays (the two in-country HIV rapid tests, the Abbott Combo assay, and the Aptima RNA assay), it is difficult to determine the HIV status of this sample.

Twelve additional samples were analyzed from individuals with acute/early infection (Table 1). All 12 samples were reactive using the Aptima RNA assay; additional assays were used

to characterize these samples (Table 1). The samples were classified as stage I ($N = 3$), III ($N = 5$) or IV ($N = 3$) using the Fiebig classification schema [16], and as stage 2 ($N = 3$) or 3 ($N = 9$) using a newer classification schema that includes fourth-generation assay test results [17]. Ten (83.3%) of the 12 samples were reactive using the Bio-Rad Combo assay. Both of the samples that were non-reactive with the Bio-Rad Combo assay had viral loads <400 copies/mL. ARV drugs (nevirapine and lamivudine) were detected in one of those samples (Table 1).

5. Conclusions

This study evaluated the performance of the Bio-Rad Combo assay for HIV diagnosis in Southern Africa. The assay had a high sensitivity for detecting established infections (100% in this study); the specificity of the assay was 96.7%, which is lower than the specificity reported in the package insert [4]. The specificity of the Bio-Rad Combo assay may be lower in other African populations and cohorts, since the HIV-negative samples tested in this study were pre-screened using a different fourth-generation assay (the Abbott Combo assay). False-positive test results may be obtained for some HIV assays due to the presence of antibodies to other infectious agents that cross react with target antigens [18–20]. Further studies are needed to determine whether co-infection with other pathogens contributes to false-positive HIV test results with the Bio-Rad Combo assay in African populations.

In this study, the Bio-Rad Combo assay detected HIV infection in 10 (83.3%) of 12 samples from individuals who were previously classified as having acute/early HIV infection. Interestingly, both of the samples that had non-reactive results using the Bio-Rad Combo assay were from individuals with viral loads <400 copies/mL. One of those samples contained no ARV drugs and was likely from an elite controller; the other sample contained ARV drugs consistent with antiretroviral treatment (ART). The recent HIV diagnostic algorithm recommended by the US Centers for Disease Control and Prevention (CDC) does not require further testing if an initial screen with a fourth-generation antigen/antibody is non-reactive. Therefore, both of these infections would have been missed in a diagnostic laboratory if the Bio-Rad Combo assay was used as the first screening assay [14]. Furthermore, both samples were classified as acute/early infections using the Fiebig classification schema and a newer classification schema that includes data from fourth generation assays [16,17]; however, these two samples were likely obtained from individuals with established HIV infection whose anti-HIV antibodies were down-regulated because they were virally suppressed. The US CDC recommendations do note that samples from people with natural or ART-induced viral suppression may produce false-negative results [14].

Viral suppression has been associated with false negative HIV tests. Elite controllers, who are virally suppressed in the absence of ART, may not be aware of their HIV status. Infection may be missed in these individuals, even if a fourth generation test is used as the first screening test [21]. Viral suppression can also occur early in HIV infection in the absence of ART. In a recent report, we described two individuals who had undetectable HIV RNA at the time of HIV seroconversion (6 months after a negative HIV test). In those cases, HIV infection was missed by one or both of the fourth-generation assays used in this report

[13]. While individuals on ART (who are aware of their HIV status) are not likely to be tested for HIV infection, this may occur in research settings and surveillance surveys (e.g., if self-reported ART data are not collected, or if participants choose not to disclose their HIV status or treatment history [22,23]). In a recent study, we analyzed samples from 101 individuals who were virally-suppressed on ART for a median of 1.6 years (range: 226 days to 6 years). All of the infections were detected using the Abbott Combo assay [21]; testing was not performed using the Bio-Rad Combo assay. In this report, we identified one individual on ART whose infection was detected using the Abbott Combo assay, but was not detected using the Bio-Rad Combo assay. Further studies are needed to assess the performance of the Bio-Rad Combo assay and other fourth-generation assays in populations that include virally suppressed individuals (elite controllers and individuals on ART). Failure to detect HIV infection in virally-suppressed persons may become increasingly important in research and surveillance studies as ART increases globally, with the expansion of treatment programs, initiation of ART at higher CD4 cell counts, and expanded use of ART for prevention of HIV transmission.

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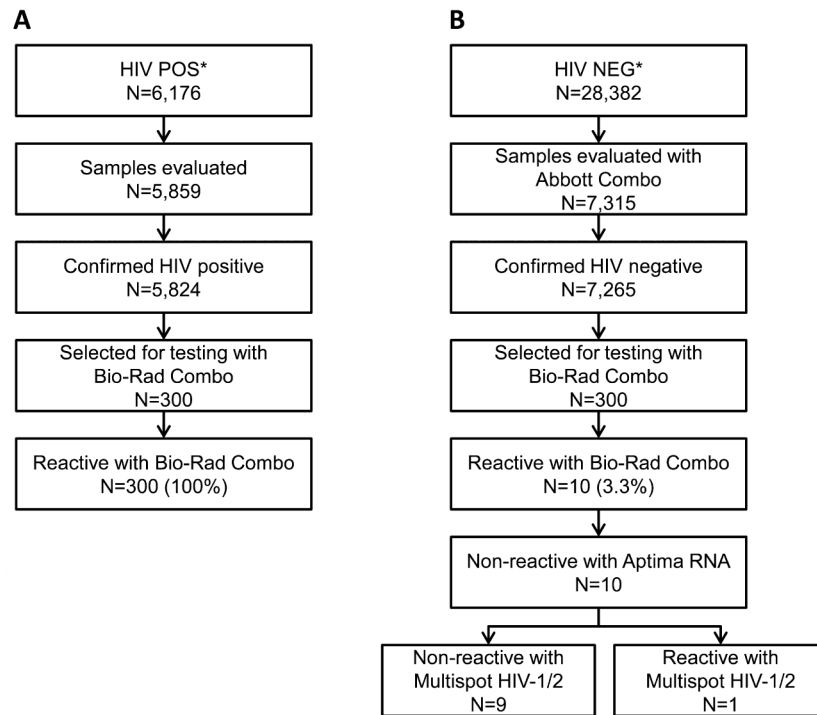


Fig. 1. Samples analyzed in this study. *HIV POS: two reactive HIV rapid antibody tests in country; HIV NEG: two non-reactive HIV rapid antibody tests in country.

Table 1

HIV assay results from individuals classified as having acute or early HIV infection.

#	Rapid test 1 ^a	Rapid test 2 ^a	Vitros EIA (3rd-gen)	Abbott Combo ^b (4th-gen)	Bio-Rad Combo ^b (4th-gen)	WB	Bands detected ^c	Aptima RNA ^d	Log ₁₀ HIV RNA ^d	ARV drugs ^e	Feibig stage ^f	4thG stage ^f
1	R	R	R	R (28.5)	R (max)	Neg	-	R	6.47	-	III	3
2	R	R	R	R (10.9)	R (11.3)	Neg	-	R	6.56	-	III	3
3	R	R	R	R (22.6)	R (12.1)	Neg	-	R	6.44	-	III	3
4	R	R	R	R (59.3)	R (max)	Ind	p24+	R	6.55	-	IV	3
5	R	R	R	R (11.4)	R (12.0)	Ind	p24+, p40+p55/51+	R	4.43	-	IV	3
6	R	R	R	R (5.5)	R (11.2)	Ind	p24+, p40+	R	4.34	-	IV	3
7	NR	NR	NR	R (14.3)	R (4.8)	Neg	-	R	5.79	-	I	2
8	NR	NR	NR	R (NA)	R (2.2)	Neg	-	R	5.18	-	I	2
9	NR	NR	NR	R (NA)	R (3.8)	Neg	-	R	5.31	-	I	2
10	NR	NR	R	R (4.1)	R (2.8)	Neg	-	R	5.38	-	III	3
11	NR	NR	R	R (NA)	NR (0.3)	Neg	-	R	BLQ (<400 copies/mL)	None	III ^f	3 ^f
12	NR	NR	R	R (NA)	NR (0.2)	Ind	p24+, p31±, p40+, p55/51+, p65±	R	BLQ (<400 copies/mL)	3TC, NVP	IV ^f	3 ^f

Abbreviations: EIA: enzyme immunoassay; gen: generation; WB: Western blot; ARV: antiretroviral; 4thG: fourth-generation; R: reactive; NR: non-reactive; Neg: negative; Ind: indeterminate; BLQ: below the limit of quantification; 3TC: lamivudine; NVP: nevirapine.

^aTwo HIV rapid tests were performed at laboratories in Africa. Other assays were performed at the HPTN Laboratory Center at Johns Hopkins University School of Medicine (Baltimore, MD, USA). Assays are described in the text.

^bSamples were tested using two fourth-generation assays: the ARCHITECT HIV Ag/Ab Combo assay (Abbott Combo) and the Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA (Bio-Rad Combo). Raw data from both assays (signal/cutoff ratios) are shown in parentheses.

^cThe bands detected are shown for samples with indeterminate Western blot results. The intensity of each band is indicated (±, +, ++).

^dSamples were tested using two HIV RNA assays: APTIMA HIV-1 RNA Qualitative Assay (Aptima RNA) and Roche COBAS AMPLICOR HIV-1 MONITOR test, v1.5. The viral load assay was performed using a validated low-volume method (lower limit of quantification: 400 copies/mL); results are reported as log₁₀ HIV RNA copies/mL with the exception of samples 11 and 12. The viral loads for these two samples were <400 copies/mL.

^eSamples were screened for the presence of 15 antiretroviral drugs using a qualitative multi-drug high resolution mass spectrometry assay.

^fThese samples were identified as being from individuals with acute or early HIV infection in a previous study [10]. The samples were characterized further in this study using additional assays, and were classified using the Feibig classification schema [16] and a classification schema that includes results from fourth-generation HIV assays [17] (Feibig stage and 4thG stage, respectively). Because the samples were collected in a single cross-sectional survey, it is not possible to determine whether samples 11 and 12 were from individuals with acute/early vs. established infection.