Failure of A Novel, Rapid Antigen and Antibody Combination Test to Detect Antigen-Positive HIV Infection in African Adults with Early HIV Infection

William Kilembe, Michelle Keeling, Etienne Karita, Shabir Lakhi, Paramesh Chetty, Matt A. Price, Heeran Makkan, Mary Latka, Morongwe Likoti, Kenneth Ilukui, Mackenzie Hurlston, Susan Allen, Gwynn Stevens, Eric Hunter

Abstract

Background: Acute HIV infection (prior to antibody seroconversion) represents a high-risk window for HIV transmission. Development of a test to detect acute infection at the point-of-care is urgent.

Methods: Volunteers enrolled in a prospective study of HIV incidence in four African cities, Kigali in Rwanda and Ndola, Kitwe and Lusaka in Zambia, were tested regularly for HIV by rapid antibody test and p24 antigen ELISA. Five subgroups of samples were also tested by the Determine Ag/Ab Combo test

Results: Of 34 group 1 samples with VL between 5x10^5 and >1.5x10^7 copies/mL (median 3.5x10^6), 1 (2.9%) was detected by the Combo antigen component, 7 (20.6%) others were positive by the Combo antibody component. No group 2 samples were antigen positive by the Combo test (0/18). Sensitivity of the Combo antigen test was therefore 1.9% (1/52, 95% CI 0.0, 9.9). One false positive Combo antibody result (1/30, 3.3%) was observed in group 4. No false-positive Combo antigen results were observed. The Combo antigen test was positive in group 6 at concentrations of 80 pg/mL, faintly positive at 40 and 20 pg/mL, and negative thereafter. The p24 ELISA antigen test remained positive at 5 pg/mL.

Conclusions: Although the antigen component of the Combo test detected antibodies to HIV earlier than the comparison antibody tests used, less than 2% of the cases of antigen-positive HIV infection were detected by the Combo antigen component. The development of a rapid point-of-care test to diagnose acute HIV infection remains an urgent goal.

Introduction

Acute HIV infection is the period following transmission of the virus and before antibody seroconversion, during which the risk of HIV transmission is high relative to chronic HIV infection [1]. Diagnosis of acute HIV infection remains a challenge. Acute retroviral syndrome symptoms may not be present, and standard detection methods such as antibody tests will fail to detect the new infection [2,3]. Diagnostic aids such as risk score algorithms and decision trees can provide some assistance to the clinician who suspects acute HIV infection [4,5], but will likely miss some cases. The use of HIV RNA tests is recommended in high incidence areas [6], however, the cost, logistics and time needed for these tests makes their widespread use impractical. The development of a point-of-care, rapid test to detect acute HIV infection is therefore important for rapid diagnosis and to allow the timely provision of risk reduction counseling to prevent onward HIV transmission, as well as the provision of treatment where the test and treat strategy becomes available. In addition, in cross sectional surveys of HIV prevalence with adequate sample size, identifying acute cases may allow investigators to estimate HIV incidence [7,8].

The Determine HIV Ag/Ab Combo test (referred to as the Combo test) is a rapid, visually read, qualitative immunochromatographic test with separate indicators for both antibody and p24 antigen results, allowing to distinguish acute from non-acute HIV infection. We present results generated by testing volunteers enrolled in a prospective study of HIV incidence in four African cities, Kigali in Rwanda and Ndola, Kitwe and Lusaka in Zambia, were tested regularly for HIV by rapid antibody test and p24 antigen ELISA. Five subgroups of samples were also tested by the Determine Ag/Ab Combo test

Citation: Kilembe W, Keeling M, Karita E, Lakhi S, Chetty P, et al. (2012) Failure of A Novel, Rapid Antigen and Antibody Combination Test to Detect Antigen-Positive HIV Infection in African Adults with Early HIV Infection. PLoS ONE 7(6): e37154. doi:10.1371/journal.pone.0037154

Editor: Philip J. Norris, Blood Systems Research Institute, United States of America

Received: November 24, 2011; Accepted: April 15, 2012; Published: June 8, 2012

Copyright: © 2012 Kilembe et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported in part by the Center for AIDS Research Virology Core (P30 AI-050409), by grants from the National Institutes of Health (R37 AI-51231, RO1 AI-64060), and in part by the generous support of the American people through the United States Agency for International Development (USAID). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

*E-mail: wkilembe@rzhrg-mail.org (WK); mkeelin@emory.edu (MK)

†These authors contributed equally to this work.
specimen panels from Rwandan and Zambian adults with or at risk for acquiring HIV infection, and a panel of serially diluted p24 antigen-positive controls.

Methods

Volunteers are followed in a study of the sexual transmission of HIV in Ndola, Kitwe and Lusaka, Zambia; and Kigali, Rwanda, as described elsewhere [9,10]; briefly, HIV-uninfected volunteers who are sexually active with a known HIV-infected partner are followed either quarterly or monthly and receive risk reduction counseling and HIV testing at each visit. Routine HIV screening for HIV-uninfected volunteers is done with the Determine rapid test (Abbott Laboratories, Chiba, Japan) and p24 ELISA (Coulter p24 HIV-1 Antigen Assay from January 2002-April 2007 (Beckman Coulter, Inc. Fullerton, California, USA) and the Vironostika HIV-1 p24 Antigen (BioMerieux bv, The Netherlands) from April 2007 to present. Confirmation of a positive Determine result was done with both second and third line rapid tests (Capillus and UniGold, Trinity Biotechnology). Additionally, acute infections were confirmed using polymerase chain reaction (PCR) on the HIV gp41 envelope region followed by genetic sequencing of the virus. Previous work with the p24 ELISA in Africa showed that the recommended assay cut-off led to a high rate of false positive results [11]. We empirically established a more conservative cut-off than either p24 ELISA or Vironostika p24 antigen assay package inserts based on the incidence of false positive results within our cohorts at the time of testing. The rate of false positive fell from 92% to 27% when the cut-offs we chose were used compared to the manufacturers cut-offs (non-published data). Therefore, only samples with results twice that of the recommended assay cut-off were read as p24 positive with the Vironostika p24 assay, and three times the recommended cut-off for the Coulter p24 assay. Plasma samples were stored at -80°C and did not exceed three freeze thaw cycles as recommended by the Determine HIV 1/2 Ag/Ab Combo Test package insert. Volunteers with confirmed HIV infection (Ag+Ab−, Ag+/Ab+, or Ag−/Ab+) were invited to join a study of early HIV infection; subjects were enrolled within 90 days of estimated date of infection (EDI). All volunteers provided informed consent, and these studies were approved by the National Ethics Committee of Rwanda, the University of Zambia Biomedical Research Ethics Committee, and the Emory University Institutional Review Board. All EC/IRBs are registered with the U.S. Office of Human Research Protection.

The testing of specimens was conducted both at the field laboratory in Lusaka, Zambia as well as the laboratory of Professor Eric Hunter at Emory University’s Vaccine Center. The field laboratory is Good Clinical Laboratory Practice (GCLP) accredited by Qualogy Inc., externally audited three times per annum by study sponsors, and participates in quarterly external quality assurance (EQA) testing schemes for all diagnostic testing. The testing of all specimens was done by three trained laboratory scientists who were all in supervisory positions within the organizations and each had greater than 4 years reading and interpreting HIV rapid testing among the Rwandan and Zambian populations. [12].

Plasma samples were retrospectively identified and tested by the Determine HIV-1/2 Ag/Ab Combo test according to the package insert (Combo test, Inverness Medical, Chiba, Japan). All results were read visually by a trained laboratory technician. Samples that produced a line in the results window that appeared to the eye fainter than the control bar were recorded by the technician as “weakly positive” but were considered positive for all analyses, as per the assay package insert. Examples of weakly positive results can be seen in the Inverness Medical report on the Combo test [13] and in [14]. Six groups of samples were tested:

1. Antigen positive, antibody negative samples from acutely HIV-infected volunteers identified between 2002 and 2009, n = 94.
2. Antigen positive, antibody positive samples from volunteers with recent HIV infection. This group includes volunteers with discrepant results across more than one antibody test (i.e., at least one antibody test was positive and at least one was negative), n = 18.
3. Antigen negative, antibody positive samples from volunteers with chronic HIV infection. Includes a convenience sample of volunteers with known HIV infection arriving for routine study visits, n = 20.
4. Antigen negative, antibody negative samples from HIV-uninfected volunteers. This group includes a convenience sample of volunteers who did not show laboratory evidence of HIV infection at this or their subsequent study visit, n = 30.
5. P24 antigen ELISA positive, antibody negative samples from HIV-uninfected volunteers (false positives), n = 25.
6. A serially diluted p24-antigen control sample at a starting concentration of 160 pg/mL (i.e., concentrations of 80, 40, 20, 10, 5, 2.5, 1.25 and 0.0625 pg/mL were tested), n = 1.

All volunteers with positive p24 ELISA results were confirmed as HIV-infected or as false positive by antibody testing at subsequent weekly visits. Confirmatory antibody testing was done using three rapid tests in accordance with both Rwanda and Zambia’s national HIV testing algorithm. Two of three antibody positive tests must be positive to yield a positive HIV antibody test result. A total of 30/34 (88.2%) antigen positive, antibody negative specimens were tested for the presence of HIV RNA (Roche Amplicor, Roche Molecular Systems) on aliquots prepared at the time of Determine HIV-1/2 Ag/Ab Combo testing. To conserve specimens, the antigen positive, antibody negative samples were diluted 20-fold with pooled and confirmed negative p24 antigen and antibody plasma. Therefore, the lower limit of detection of the assay was 8,000 copies/mL. The upper limit of the assay was 15x10⁶ copies/mL. HIV-1 subtyping was done from amplified gag, pol or gag/1 sequences using the Recombination Identification Program [http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html] as described previously [15].

The results of the Combo antigen test are compared to the results of the p24 antigen ELISA to calculate test sensitivity. Poisson exact methods were used to calculate 95% confidence intervals. Analyses were conducted with Stata Statistical Software (Release 11, College Station, TX, USA: StataCorp LP) and SAS Statistical Software (SAS Institute Inc. 2009, Cary, NC).

Results

Study Population

A total of 135 plasma samples from 123 volunteers were tested, including 99 volunteers from Lusaka, Zambia, 14 from the Zambia’s Copperbelt (Kitwe and Ndola), and 10 from Kigali, Rwanda (Table 1). Of the volunteers tested, 78 (63%) were HIV-infected (groups 1-3), and 45 (37%) were HIV-uninfected controls (groups 4 and 5). The volunteers included 58 (47%) women. Nine volunteers provided multiple samples drawn sequentially, including two with HIV infection. One volunteer with HIV infection provided two samples (one Ag+/Ab−; and one Ag+/Ab+) and the second seroconverting volunteer provided two samples (one Ag+/Ab−; and one Ag−/Ab+). The remaining seven volunteers who provided multiple samples were all from group 5 (p24 ELISA false
positive volunteers), including four who provided two samples, and three who provided three samples (Table 1). Subtype data were available for 54/78 (69.2%) of the volunteers with HIV infection (Table 1). In Zambia of the 45 for whom subtype was available, 44 (97.8%) were infected with subtype C, and 1 (2.2%) with a subtype A/C recombinant virus. In Rwanda, 7/9 (77.8%) were infected with subtype A1, and 2 (22.2%) with subtype C.

**Combo Antigen and Antibody Results**

Of 34 samples from acutely infected volunteers in group 1 (antigen positive, antibody negative), one sample (2.9%, from a subtype-C infected volunteer) was antigen positive, antibody negative by the Combo test, and seven (20.6%) additional samples were antibody positive but antigen negative (Table 2). Of 18 samples with both detectable p24 antigen and antibody from group 2, no samples were antigen positive and all were antibody positive by the Combo test. Therefore, among all 52 HIV positive samples with a positive antigen ELISA result, the sensitivity of the Combo antigen test was 1.9% (95% confidence interval: 0.0, 9.9). The single antigen positive result from the Combo test was weakly positive, with the antigen band appearing fainter than the control band upon visual inspection.

The univariate summary statistics for p24 antigen concentration among all ELISA identified p24 positives and viral load values are reported in Table 3. The difference in p24 antigen concentration between groups 1 and 2 (Ag+/Ab− and Ag+/Ab+) was not statistically significant p = .1661. Likewise, the concentration of p24 antigen in the 8 specimens that the rapid antigen/antibody was able to identify (one Ag+ and seven Ab+) versus the 26 ELISA p24 positive specimens that tested negative on the rapid assay did not differ with significance, p = .9031.

Viral load data were available from 37 (71.2%) of the group 1 and 2 samples. The median HIV RNA concentration was 3.5x10^6 copies/mL, with 8 values above the assay upper limit of 15x10^6 copies/mL. The sample positive by Combo antigen had a viral load of 1.1x10^7 copies/mL and was higher than 66% of the group 1 and 2 samples with viral load data. There was a strong correlation between p24 antigen concentration and viral load for Ag+/Ab− individuals (r = 0.741, p < .0001) as well as combined Ag+Ab− and Ag+Ab+ (r = 0.722, p < .0001).

**Table 1. Study Population Characteristics.**

| Characteristic                  | Group 1: Ag+ Ab− | Group 2: Ag+Ab+ | Group 3: Ag− Ab+ | Group 4: Ag− Ab− | Group 5: Ag False Ab− |
|--------------------------------|------------------|-----------------|------------------|------------------|----------------------|
| Total number of volunteers     | 34               | 100             | 18               | 100             | 28                   | 100                  | 30               | 100             | 15               | 100             |
| Sex                            |                  |                 |                  |                  |                      |                     |                  |                  |                  |                  |
| Female                         | 21               | 61.8            | 4                | 23.3            | 19                   | 67.9                | 8                | 26.7            | 6                | 40               |
| Male                           | 13               | 38.2            | 14               | 77.7            | 9                    | 32.1                | 22               | 73.3            | 9                | 60               |
| Enrolment site                 |                  |                 |                  |                  |                      |                     |                  |                  |                  |                  |
| Kigali, Rwanda                 | 5                | 14.7            | 4                | 22.2            | 0                    | −                   | 0                | −               | 1                | 7                |
| Lusaka, Zambia                 | 23               | 67.7            | 8                | 44.4            | 28                   | 100                 | 30               | 100             | 12               | 80               |
| Copperbelt, Zambia             | 6                | 17.6            | 6                | 33.3            | 0                    | −                   | 0                | −               | 2                | 13               |
| HIV-1 subtype                  |                  |                 |                  |                  |                      |                     |                  |                  |                  |                  |
| A1                             | 4                | 11.8            | 3                | 16.7            | 0                    | −                   | 0                | −               | 0                | −                |
| C                              | 29               | 85.3            | 15               | 83.3            | 4                    | 14.3                | 0                | −               | 0                | −                |
| A/C recombinant                | 1                | 2.9             | 0                | −               | 24                   | 85.7                | 0                | −               | 0                | −                |
| Missing                        | 0                | −               | 0                | −               | 0                    | −                   | 0                | −               | −                | 30               | 100             | 15               | 100             |
| Not Applicable                 | 0                | −               | 0                | −               | 0                    | −                   | 0                | −               | −                | 30               | 100             | 15               | 100             |
| HIV-1 viral load*              |                  |                 |                  |                  |                      |                     |                  |                  |                  |                  |
| Number tested                  | 30               | 88.2            | 7                | 38.9            | 0                    | −                   | 0                | −               | 0                | −                |
| Median, (IQR)                  | 6.8 (6.2,7.2)    | 6.2 (6.1,6.8)   |                  |                  |                      |                     |                  |                  |                  |                  |
| Number of samples contributed**|                  |                 |                  |                  |                      |                     |                  |                  |                  |                  |
| 1                              | 34               | 100             | 18               | 100             | 28                   | 100                 | 30               | 100             | 8                | 47               |
| 2                              | 0                | −               | 0                | −               | 0                    | −                   | 0                | −               | 4                | 33               |
| 3                              | 0                | −               | 0                | −               | 0                    | −                   | 0                | −               | 3                | 20               |

*Log10 transformed viral copies/mL.
**7/123 volunteers contributed more than one sample to their respective group from different dates (group 5) and 2 volunteers from group 1 contributed a second sample; one to group 2 and the other to group 3.

doi:10.1371/journal.pone.0037154.t001
None of the group 4 (n = 30) or group 5 (n = 25) samples from volunteers without HIV infection were antigen-positive by the Combo test. However, one group 4 sample (3.3%) was antibody-positive by the Combo test, a false positive result. In a comparison of the Combo test antigen component to the ELISA antigen test, both assays were run against serially diluted p24 antigen control provided in the Vironostika HIV-1 Antigen ELISA kit, in which the positive control is serial diluted with the kit’s negative control per testing instructions (group 6). The reported optical density of the serial p24 dilution was the average of duplicate runs. The Combo antigen test remained positive to p24 antigen concentrations of 20 pg/mL and the ELISA remained positive to 5 pg/mL (Table 4).

Discussion

In this prospective cohort of African adults infected with or at high risk for HIV infection, the Determine Ag/Ab Combo test detected only 8 of 34 (23.5%) persons with acute HIV infection. Only one of these 8 positive Combo test results was scored as antigen-positive, the remaining 7 were antibody-positive. Also, the antigen component of the Combo test did not detect any of the 18 samples that were antigen- and antibody-positive in volunteers with early HIV infection, and when tested against serially diluted p24 antigen-positive controls, was not as sensitive as the p24 ELISA. While the antibody component of the Combo test was able to detect antibodies to HIV before the rapid HIV tests used in this report, the antigen component of the Combo test was much less sensitive than the p24 antigen ELISA in detecting acute infection in these subtype A and C plasma samples. The Combo test package insert reports two analytical sensitivity studies, one which used an HIV Ag panel from the French Blood Establishment and the second used purified HIV-1 p24 native protein. The reported lower limits of p24 antigen concentration that could be detected by the Combo assay were 25 pg/mL and 12.5 pg/mL respectively [16]. Twenty one percent (11/52) of the acute infections we identified with the p24 Antigen ELISA had concentrations less than 25 pg/mL but of these 11, only 2 had p24 Antigen concentrations less than 12 pg/mL and theoretically should have been picked up by the Combo assay. The sole acute infection that was detected by the Combo assay had a p24 antigen concentration of 248.3 pg/mL. Recent work in Malawi, where the HIV epidemic is primarily subtype C, also found that the Combo test failed to detect acute HIV infection. The authors report that 8

---

**Table 2. Performance of the Determine Ag/Ab Combo test.**

| Determine Ag/Ab Combo test results | Group 1: Ag+ Ab− | Group 2: Ag+ Ab+ | Group 3: Ag− Ab+ | Group 4: Ag− Ab− | Group 5: Ag False+ Ab− |
|-----------------------------------|------------------|------------------|------------------|------------------|-------------------------|
| Total number of samples           | 34               | 18               | 28               | 30               | 25                      |
| Ag negative, Ab negative          | 26               | 0                | 0                | 29               | 25                      |
| Ag positive, Ab positive          | 0                | 0                | 0                | 0                | 0                       |
| Ag positive, Ab negative          | 0                | 0                | 0                | 0                | 0                       |
| Ag weak pos, Ab negative          | 1                | 2.9              | 0                | 0                | 0                       |
| Ag negative, Ab positive          | 1                | 2.9              | 15               | 83.3             | 0                       |
| Ag negative, Ab weak pos          | 6                | 17.6             | 3                | 16.7             | 0                       |

Group 1: Antigen positive, antibody negative acute HIV infection (Ag+Ab−).
Group 2: Antigen positive, antibody positive early HIV infection (Ag+Ab+).
Group 3: Antigen negative, antibody positive chronic HIV infection (Ag−Ab+).
Group 4: Antigen negative, antibody negative volunteers without HIV infection (Ag−Ab−).
Group 5: Antigen false positive, antibody negative volunteers without HIV infection (Ag−Ab−).

Table 3. ELISA p24 Antigen Concentration and Viral Load Summary Statistics.

| p24 Ag Concentration (pg/ml) | Group 1 & 2 | Group 1 | Group 1 Subset Rapid Test NEG | Group 1 Subset Rapid Test POS (Ag or Ab) | Group 2 | Viral Load (copies/ml) |
|------------------------------|-------------|---------|-------------------------------|------------------------------------------|---------|-----------------------|
| N                            | 52          | 34      | 26                            | 8                                        | 18      | 37                    |
| Mean                         | 126.01      | 138.7   | 136.2                         | 146.8                                    | 102.1   | 7,013,211             |
| Median                       | 94.1        | 140.4   | 140.4                         | 162                                      | 50.4    | 6,316,740             |
| IQR (75%,25%)                | (201,2,32.7)| (203,5,48.4) | (193,51)                    | (267,8,14,4)                             | (152,7,15.7) | (12950000, 1706540) |
| Min,Max                      | (8,2,457.8)| (8,2,457.9) | (14,2,457.9)                | (8,2,277.7)                              | (10,4,351.2) | (545720,150000001)   |

*Upper limit of detection for the VL assay = 15,000,000, any observation that exceeded 15,000,000 recorded as 15,000,001.

Group 1: Antigen positive, antibody negative i.e. acute HIV infection (Ag+Ab−).
Group 2: Antigen positive, antibody positive i.e. early HIV infection (Ag+Ab+).

doi:10.1371/journal.pone.0037154.t003
cases of acute HIV infection were missed by the antigen component of the Combo test (although 2 were detected by the antibody component), and that 14 false positive Combo antigen results were observed in 838 HIV-uninfected persons, for a sensitivity of 0.00 and a specificity of 0.98 [17]. A report from France to evaluate the sensitivity of five rapid HIV tests in a health care setting found the Combo test did not detect p24 antigen in two volunteers with recent HIV infection. The concentration of p24 antigen in the plasma of one volunteer was 380 pg/mL, and undetectable in the other [18]. In contrast, the assay has been tested in San Francisco, and correctly identified all persons with chronic infection, and 7/9 persons with acute HIV infection [19].

Another recent report found the sensitivity of the Combo test in a bank of acute HIV infection specimens from Holland to be 86.6% (32/39 samples, 95% CI: 76.0, 93.7). However two of the 32 samples were reactive in the antibody component only and no subtype or demographic data are given. The authors of this work also report being able to detect antigen in cell supernatants from subtypes A, B, C, D, CRF_01AE, CRF01_AG, G, G/H, J, G/A, and K (3-5 samples of each) diluted in negative human plasma, however the concentration of p24 antigen in each sample was not reported [20].

While the package insert for the Combo test states that specimens that have been freeze-thawed more than three times should not be used for testing, a majority of our samples tested had not been thawed more than once. Moreover, the specimen in this report that was antigen-positive by both p24 antigen ELISA and Combo test was 3.5 years old at the time of testing. None of the 31 more recent group 1 or 2 samples were detected by the antigen component of the Combo test, including four samples that were drawn within 2 months of testing. Finally, all viral load assays on group 1 and 2 samples were performed on aliquots processed in parallel to those tested in the Combo test, and so it is unlikely that freeze-thawing of samples contributed to the lack of reactivity in this assay.

**References**

1. Cohen MS, Shaw GM, McMichael AJ, Haynes BF (2011) Acute HIV-1 Infection. N Engl J Med 364: 1943–1954.

2. Pilcher CD, Christopoulos KA, Golden M (2010) Public health rationale for rapid nucleic acid or p24 antigen tests for HIV. J Infect Dis 201 Suppl 1: S7–15.

3. Sullivan PS, Fidelis U, Wall KM, Chomba E, Vwalika C, et al. (2011) Prevalence of seroconversion symptoms and relationship to set point viral load: Findings from a subtype C epidemic, 1995–2009. AIDS In Press.
4. Powers KA, Miller WC, Pilcher CD, Mapanje C, Martinson FE, et al. (2007) Improved detection of acute HIV-1 infection in sub-Saharan Africa: development of a risk score algorithm. AIDS 21: 2237–2242.

5. Zetola NM, Pilcher CD (2007) Diagnosis and management of acute HIV infection. Infect Dis Clin North Am 21: 19–48.

6. CDC (2003) Advancing HIV prevention: new strategies for a changing epidemic—United States, 2003. MMWR Morb Mortal Wkly Rep 52: 329–332.

7. Petersen LR, Saten GA, Doddi R, Busch M, Krakowman S, et al. (1994) Duration of time from onset of human immunodeficiency virus type 1 infectiousness to development of detectable antibody. The HIV Seroconversion Study Group. Transfusion 34: 283–289.

8. Brookmeyer R, Quinn TC (1995) Estimation of current human immunodeficiency virus incidence rates from a cross-sectional survey using early diagnostic tests. Am J Epidemid 141: 166–172.

9. Dunkle KL, Stephenson R, Karita E, Chomba E, Kayitenkore K, et al. (2000) New heterosexually transmitted HIV infections in married or cohabiting couples in urban Zambia and Rwanda: an analysis of survey and clinical data. Lancet 371: 2183–2191.

10. Kempf MC, Allen S, Zulu I, Kancheva N, Stephenson R, et al. (2008) Enrollment and retention of HIV discordant couples in Lusaka, Zambia. J Acquir Immune Defic Syndr 47: 116–125.

11. Mulenga J, Hunter E, Manigart O, Stevens G, Allen S, et al. (2006) P24 antigen screening for early detection of HIV infection in discordant heterosexual couples in Zambia. Amsterdam, The Netherlands.

12. Borrazo DH, Luisi N, Karita E, McKinsey S, Sharkey T, et al. (2011) Indeterminate and discrepant rapid HIV test results in couples’ HIV testing and counselling centers in Africa. J Int AIDS Soc 14: 18.

13. Inverness Medical. Determine HIV-1/2 Ag/Ab Combo: a revolutionary fourth generation rapid HIV test.

14. Keren T, Guidasci T, Rivetz B, Fish F. Performance evaluation of the Determine HIV-1/2 Ag/Ab Combo, a novel 4th generation rapid test; 2009 July 19–22, 2009; Cape Town, South Africa.

15. Trask SA, Derdeyn CA, Fideli U, Chen Y, Meleth S, et al. (2002) Molecular epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia. J Virol 76: 397–405.

16. Determine HIV-1/2 Ag/Ab Combo Test Package Insert v1.1Feb12. Available: http://www.determinetest.com/pdf/DetermineHIV Combo Pack Insert v2 20100302009.pdf. Accessed 2012 May 14.

17. Kempf MC, Allen S, Zulu I, Kancheva N, Stephenson R, et al. (2011) Poor Performance of Determine HIV 1/2 Ag/Ab Combo Assay for the Detection of Acute HIV Infection in Lilongwe, Malawi [654]. Boston, MA, USA.

18. Pavie J, Rachline A, Loze B, Niedbalski L, Delaugerre C, et al. (2010) Sensitivity of five rapid HIV tests on oral fluid or finger-stick whole blood: a real-time comparison in a healthcare setting. PLoS One 5: e11581.

19. Pilcher CD, Louie B, Keating S, Pandori M, Fish F, et al. (2009) A Clinical Study of Antigen/Antibody Rapid Testing for Acute HIV Infection. Montreal, Canada.

20. Rechert G, Fransen K (2010) Evaluation of a rapid and simple fourth-generation HIV screening assay for qualitative detection of HIV p24 antigen and/or antibodies to HIV-1 and HIV-2. J Virol Methods 166: 218–222.

21. Fideli US, Allen SA, Muonoda R, Trask S, Hahn BH, et al. (2001) Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. AIDS Res Hum Retroviruses 17: 901–910.

22. Schuppach J (2003) Viral RNA and p24 antigen as markers of HIV disease and antiretroviral treatment success. Int Arch Allergy Immunol 132: 196–209.

23. Fiscus SA, Cheng B, Crowe SM, Demeter L, Jennings C, et al. (2006) HIV-1 viral load assays for resource-limited settings. PLoS Med 3: e417.