Clinical Application Evaluation of Two Fourth-Generation Human Immunodeficiency Virus (HIV) Screening Assays in West China Hospital

Yongming Liu, Dongdong Li, Tingting Wang, Kening Yan, Siyuan Zhu, Tingfu Yang, Lan Luo, and Chuanmin Tao*

Division of Clinical Microbiology, Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China

Background: Fourth-generation human immunodeficiency virus (HIV) screening assays have been used in many laboratories. The Elecsys® HIV combi PT assay is a new kind of fourth-generation HIV screening assay developed to allow earlier detection of seroconversion. **Methods:** A total of 271,845 routine specimens were detected using the Elecsys® HIV combi assay and Elecsys® HIV combi PT assay from September 2010 to December 2012 in a large university hospital. Repeatedly, reactive screening samples were confirmed according to recommended confirmatory algorithms. **Results:** The false-positive rate and positive predictive value (PPV) of two assays are 0.08 and 78.35%, respectively, for the Elecsys® HIV combi assay and 0.07 and 82.21% for the Elecsys® HIV combi PT assay. Ninety-

four percent cases with cutoff index ratio <15.0 were false-positive. When we set the specificity as 95.0 and 99.0%, PPV could increase to 98.7, 99.6, 98.8, and 99.7%, and sensitivity reduced to 99.2, 98.4, 98.5, and 96.8% for the Elecsys® HIV combi assay and the Elecsys® HIV combi PT assay, respectively. **Conclusions:** The Elecsys® HIV combi PT assay shows a better performance in specificity than the Elecsys® HIV combi assay. Most weakly reactive results were false-positive, this means it still need to be improved and it will need laboratory personnel to communicate with the clinical doctor and patients more properly about the result of the assay. J. Clin. Lab. Anal. 29:146–152, 2015. © 2014 Wiley Periodicals, Inc.

Key words: HIV; screening; specificity; sensitivity; evaluation

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) remains the most devastating outcome of human immunodeficiency virus (HIV) infection. It has had a profound effect on human illness and death over the last 30 years and has close to a 100% fatality rate in untreated patients. Data from Montreal indicate that 50% of HIV transmission is attributable to recently infected persons (1). The laboratory plays a key role in preventing the spread of this epidemic and laboratory-based methods have undergone tremendous change. Universal screening to identify HIV infection is recommended so that infected individuals can be linked to care, antiretroviral medications, and prophylaxis against opportunistic infections (2). The recent approval of fourth-generation HIV tests, which combine detection of HIV antibodies with HIV antigens, has forced many laboratories to weight the costs and benefits of tran-

sitioning to these tests (3). At least two kinds of assays or reagents must be used in HIV screening work according to National Guideline for Detection of HIV/AIDS (2009 edition) (4).

The Elecsys® HIV combi PT assay is a fourth-generation antigen–antibody combination assay developed to allow earlier detection of seroconversion, and includes a pretreatment step to improve specificity and

Grant sponsor: National Natural Science Foundation of China; Grant number: 81072432; Grant sponsor: Science and Technology Support Program of Sichuan Province; Grant number: 2012FZ0126.

*Correspondence to: Chuanmin Tao, Department of Laboratory medicine, West China Hospital, Sichuan University, 37 Guoxue Alley, Sichuan, Chengdu 610041, China. E-mail: taochuanmin@sina.com

Received 19 July 2013; Accepted 8 January 2014

DOI 10.1002/jcla.21743

Published online in Wiley Online Library (wileyonlinelibrary.com).

increase sensitivity to HIV-1 p24 antigen compared with the Elecsys® HIV combi assay. The Elecsys® HIV combi PT assay has been reported to compare favorably with other kind of fourth-generation assay of different brand in analytical sensitivity for HIV-1 p24 antigen, commercial seroconversion panels, and HIV subtypes (5,6). In the laboratory of West China Hospital, the fourth-generation assay (the Elecsys® HIV combi PT assay, electrochemiluminescence immunoassay (ECLIA)) has been used to replace another fourth-generation assay (the Elecsys® HIV combi assay, electrochemiluminescence immunoassay (ECLIA)). The clinical diagnostic features of these two assays should be known by technologists who are always asked to interpret the discordant results (7) and whether the cutoff index (COI; signal sample/cutoff) ratios can be predictive of confirmed HIV infection, as in HCV and HBV works (8–10).

As with all infectious assays, nonspecific binding can occur, and false-positive results can be found, especially in weakly positive samples (11). In the case of HIV infection, Western blotting and RNA testing were needed to confirm HIV infection. As expected, it is found that most weakly reactive or borderline results were negative when determined by the Western blot (WB) and RNA tests.

In this application evaluation, 271,845 HIV test results from laboratories operated by West China Hospital under routine conditions were included to evaluate the performance of this fourth-generation HIV screening assays.

MATERIALS AND METHODS

Ethics Statement

The observational study received ethical approval from West China Hospital of Sichuan University. The study was based on existing laboratory data collected at laboratory of West China Hospital and the data were analyzed anonymously.

Screening Systems Procedures

Two fully automated analyzers were compared, the Elecsys® HIV combi assay on the Cobas e 601 and MODULAR E170 analyzers (Roche Diagnostics, Mannheim, Germany). Testing data were retrospectively collected from plasma specimens in West China Hospital (a university hospital with 4,300 beds) without personal identifiers, with a time range from September 2010 to December 2012. Until March 2012, screening for HIV infection was carried out with a fourth-generation immunoassay (the Elecsys® HIV combi assay on the Cobas and MODULAR analyzers). Since April 2012, an updated fourth-generation immunoassay (the Elecsys® HIV combi PT assay on the Cobas and MODULAR analyzers) has been used at our laboratory for both diagnosis and screening

purposes. They were conducted according to manufacturer's instructions.

The result of a sample is given either as reactive or non-reactive as well as in form of a COI. The analyzer automatically calculates the cutoff based on the measurement of calibrators. Samples having a COI in the range ≥ 0.90 to < 1.0 are considered borderline and ≥ 1.0 are considered reactive in tests. All initially reactive or borderline samples have been retested in duplicate and considered repeatedly reactive if its retest result is reactive. Repeatedly reactive samples will be confirmed according to recommended confirmatory algorithms. Confirmatory tests include WB and HIV RNA tests. Patients who do not want to accept the confirmatory test in our laboratory, their results are missed. The operational flow chart and comparison of the results obtained by two HIV screening assays is shown in Fig. 1.

HIV Confirmatory Tests

HIV confirmatory tests include WB HIV blot 2.2 (MP Diagnostics, Singapore) and COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Diagnostics). In China, HIV WBs are interpreted following the National Guideline for Detection of HIV/AIDS (2009 edition) (4), which require detection p24, gp41/160, and gp120 for positive results. The absence of HIV-specific bands is a negative result. HIV-specific bands that are present but do not meet the criteria for positivity is an indeterminate pattern. HIV results of individual samples were recorded after reading by two different laboratory technologists and according to manufacturer's criteria for interpretation of positive, negative, or inconclusive results. Infection with HIV-2 was not found in our setting.

For COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Diagnostics), the concentration of HIV-1 RNA in EDTA plasma that can be detected with a positive rate of greater than 95% as determined by Probit Analysis is 48 copies/ml. It was found to give a linear response from 48 copies/ml to 1×10^7 copies/ml applying the accuracy acceptance criterion of $\pm 0.3 \log_{10}$ from the nominal input concentration.

Statistical Analysis

Most of the data were from our clinical laboratory information system. Some follow-up data were searched from Hospital Information System. Categorical variables were compared by chi-square test. The statistical software package SPSS 11.0 (SPSS Inc., Chicago, IL) was used for data analysis. Repeated data were excluded by patient ID. The data were reviewed by laboratory quality control staff, and data entry was performed by two independent persons.

Fig. 1. The operational flow chart and comparison of the results obtained by two HIV screening assays.

RESULTS

As indicated above, the result of a sample is given in the form of COI. During the analysis period, of those 271,845 specimens, 172,367 (63.41%) were carried out with the Elecsys[®] HIV combi assay, 99,478 (36.59%) with the Elecsys[®] HIV combi PT assay. Of the 270,811 specimens with interpretable HIV test results, 514 (0.30%) of 171,690 using the Elecsys[®] HIV combi assay and 342 (0.35 %) of 99,121 using the Elecsys[®] HIV combi PT assay were HIV positive (samples with repeatedly reactive screening assays, but follow-up confirmation tests not performed were excluded). The false-positive rate (FPR) and

positive predictive value (PPV) of two assays are 0.08 and 78.35% for the Elecsys[®] HIV combi assay and 0.07 and 82.21% for the Elecsys[®] HIV combi PT assay as shown in Table 1.

Only 243 WB-discordant samples (WB test result is indeterminate or negative) were followed up by WB test 4 weeks later and/or RNA tests of subsequent samples of the same patients, others were lost to follow-up. Two hundred sixteen cases turned out to be negative and 27 cases turned out to be positive as shown in Table 2.

The mean COIs are shown in Table 3, which suggested that the number of cases with HIV infection increased in

TABLE 1. FPR and PPV of Two Different Screening Assays^a

	TP	FP	TN	Sen% (95% CI)	Sp% (95% CI)	FPR% (95% CI)	PPV% (95% CI)
HIV combi assay	514	142	171,034	100 (100–100)	99.92 (99.91–99.92)	0.08 (0.07–0.09)	78.35 (75.19–81.51)
HIV combi PT assay	342	74	98,705	100 (100–100)	99.93 (99.91–99.95)	0.07 (0.05–0.09)	82.21 (78.54–85.88)

TP, true-positive; FP, false-positive; TN, true-negative; Sen, sensitivity; Sp, specificity; FPR, false-positive rate; PPV, positive predictive value; CI, confidence interval.

^aSamples with repeatedly reactive screening assays, but confirmation tests not performed or confirmation tests result cannot be defined as positive or negative were excluded.

TABLE 2. Follow-up of 243 WB-Discordant Samples

WB test→follow-up test	Elecsys [®] HIV combi assay	Elecsys [®] HIV combi PT assay
WB IND→WB POS	5	3
WB IND→WB NEG	6	12
WB IND→RNA POS	9	9
WB IND→RNA NEG	25	23
WB NEG→RNA POS	1	0
WB NEG→RNA NEG	111	39

WB, Western blot; RNA, HIV RNA test; POS, positive; NEG, negative; IND, indeterminate.

TABLE 3. Median and Extreme COI Ratios of Different Confirmed HIV Results Samples

Confirmed HIV results	Median and extreme COI ratios of screening test	
	Elecsys [®] HIV combi assay	Elecsys [®] HIV combi PT assay
WB POS	312.75 (2.59, 1712.00)	397.25 (5.65, 2719.00)
WB NEG	3.90 (1.05, 58.73)	1.60 (1.06, 39.67)
WB IND	156.6 (12.14, 765.30)	6.98 (1.52, 225.60)

WB, Western blot; POS, positive; NEG, negative; IND, indeterminate; COI, cutoff index.

relation to the COI ratio (Table 4). In cases with COI ratio <15.0, there were three cases that showed positive results for the Elecsys[®] HIV combi assay and four cases for the Elecsys[®] HIV combi PT assay by confirmatory tests. Most of the cases with COI between 0.9 and 49.99 turned out to be negative when determined by WB with or without RNA tests, 94.6% (140/148) for the Elecsys[®] HIV combi assay and 85.1% (74/87) for the Elecsys[®] HIV combi PT assay (Table 4).

In repeatedly reactive samples with follow-up confirmatory tests results, we set the specificity as 95.0% by setting artificial cutoff value as 19.1 for the Elecsys[®] HIV combi assay according to the Receiver Operating Characteristic (ROC) curves. Accordingly, the PPV could be increased to 98.7%, and sensitivity reduced to 99.2%, respectively. Likewise, we set the artificial cutoff value as 50.5 to set the specificity as 99.0% for the Elecsys[®] HIV combi assay according to the ROC curves and

TABLE 4. Confirmation Tests Results in Relation to COI ratios^a

COI ratio	No. of cases in each group		
		Positive (%)	Negative (%)
Elecsys [®] HIV combi assay			
0.9–14.99	138	3 (2.2)	135 (97.8)
15–49.99	10	5 (50.0)	5 (50.0)
50–99.99	15	13 (86.7)	2 (13.3)
100–199.99	94	94 (100.0)	0 (0.0)
200–399.99	229	229 (100.0)	0 (0.0)
400–799.99	151	151 (100.0)	0 (0.0)
>799.99	19	19 (100.0)	0 (0.0)
Total	656	514 (78.4)	142 (21.6)
Elecsys [®] HIV combi PT assay			
0.9–14.99	74	4 (5.4)	70 (94.6)
15–49.99	13	9 (69.2)	4 (30.8)
50–99.99	21	21 (100.0)	0 (0.0)
100–199.99	57	57 (100.0)	0 (0.0)
200–399.99	80	80 (100.0)	0 (0.0)
400–799.99	87	87 (100.0)	0 (0.0)
>799.99	84	84 (100.0)	0 (0.0)
Total	416	342 (82.2)	74 (17.8)

COI, cutoff index.

^aExclude samples with repeatedly reactive screening assays, but the confirmation tests not performed or completed.

TABLE 5. Performance of Two Assays in Repeated Reactive Samples According to Cutoff Index

Parameter	Elecsys [®] HIV combi assay		Elecsys [®] HIV combi PT assay	
Specificity (%)	95.0	99.0	95.0	99.0
Value of COI	19.1	50.5	18.6	39.5
Sensitivity	99.2	98.4	98.5	96.8
(95% CI)	(98.4–100.0)	(97.0–99.8)	(97.2–99.8)	(94.3–99.3)
PPV (%)	98.7	99.6	98.8	99.7
(95% CI)	(97.7–99.7)	(98.9–100.3)	(97.7–99.9)	(98.9–100.5)

CI, confidence interval; PPV, positive predictive value.

PPV accordingly increased to 99.6%, the sensitivity reduced to 98.4%. For the Elecsys[®] HIV combi PT assay, we set the specificity as 95.0% by setting artificial cutoff value as 18.6 and the PPV accordingly increased to 98.8%, the sensitivity reduced to 98.5%. When we set the

specificity as 99.0% by setting artificial cutoff value as 39.5 for the Elecsys® HIV combi PT assay, the PPV could be increased to 99.7%, the sensitivity reduced to 96.8% accordingly (Table 5).

DISCUSSION

This study is an attempt to evaluate the clinical diagnosis performance of two immunoassays for HIV screening and analyze the relationship between the COI ratios and confirm HIV infections in the context of highly complex region of China. Previous studies (12) showed that high Signal-to-Cutoff (S/CO) ratio were predictive of confirmed positive results, therefore the aim of this study was to analyze the extent of COIs overlap between false-positive and confirmed positive results to determine the optimal cutoff, which is reliable predictive of the final status of HIV infection. As shown in Table 1, the FPR and specificity of repeatedly reactive sample in the Elecsys® HIV combi PT assay is 0.07 and 99.93%, which seemed better than that of the Elecsys® HIV combi assay though without statistic significance. The PPV of the Elecsys® HIV combi PT assay is 82.21%, which is higher than the Elecsys® HIV combi assay. The Elecsys® HIV combi PT assay shows a better performance in specificity than the Elecsys® HIV combi assay, as the former includes a pretreatment step to improve specificity and increase sensitivity to HIV-1 p24 antigen, which may require different cutoff thresholds for optimal specificity and sensitivity in a single test (13, 14). This result agrees with a former study that finds out that the specificity of the Elecsys® HIV combi PT assay (99.5%) is higher than the Elecsys® HIV combi assay (99.0%) at the same sensitivity (100%), though without statistical difference ($P > 0.05$) (6). Some of these assays, however, did not have any advantage over the HIV antigen-only assays (15), the specificity of the early combination assays was not significantly different from the specificity observed for HIV antibody-only assays (16, 17). However, the Elecsys® HIV combi PT assay was more sensitive and showed a higher PPV than the Elecsys® HIV combi assay, for it is of importance for screening assay to improve sensitivity and avoid missed diagnosis, which can shorten windows period.

By setting the specificity as 95, 99% in the repeatedly reactive samples, we tried to compare the sensitivity of these screening assays. The sensitivity of the Elecsys® HIV combi PT assay (98.5, 96.8%) is lower than that of the Elecsys® HIV combi assay (99.2, 98.4%), though there is no statistical difference ($P > 0.05$). Only 1,072 cases reactive samples with follow-up confirmatory tests were included in this comparison, a further research is needed.

With numerous technical improvements, the current serological HIV screening assays were more sensitive com-

pared to the early immunoassay detecting the HIV antibody (18). Since 2001, new “fourth-generation” HIV tests designed to detect both HIV p24 antigen and antibody in a single immunoassay have shortened the diagnostic window with additional sensitive HIV antigen tests. Currently, these new fourth-generation assays are widely used for routine laboratory diagnosis of HIV. However, it should be recognized that no laboratory test is 100% accurate, and results in consistent with clinical finding should be repeated or assessed with different tests. Even false-positive WB has been reported (19). False positives in the retrospective evaluation may have been true positives due to the inability of WB to detect acute and/or recent infections (20). Despite time consuming, subjective, and expensive, the WB assay has remained the principal confirmatory assay that detects a number of conserved protein regions produced by the virus.

Laboratory diagnosis of HIV infection is generally based on demonstration of anti-HIV antibodies and in some discordant cases detection of viral antigens and RNA. As we found in our research, there was one patient with a repeatedly reactive screening test result, but with a negative WB result and positive RNA result ($>10^7$ copies/ml). This patient's sex partner had been infected with HIV and had a positive WB result. This patient was diagnosed with full-blown AIDS and died before she can accept the second time WB test. The fourth-generation of HIV screening test is detecting both anti-HIV antibodies and P24 antigen of HIV, but the WB test can only detect the anti-HIV antibodies. Therefore, it is important to take this in mind to avoid misdiagnosis for laboratory diagnosis of HIV infection and HIV RNA test is needed as a supplemental test. However, nucleic acid test (NAT) remains unaffordable in many countries, is time-consuming and labor intensive, and its cost benefit in low-prevalence countries is under review (21). Such strategies have already been implemented in several countries as the primary method for screening (22).

The improved sensitivity was highly desirable to avoid time-consuming and expensive confirmatory testing, as well as to eliminate delays in reporting results and avoid unnecessary deferrals. The benefit of an HIV combination assay in urban centers with high sample throughput was described in an earlier study (23). To be useful, diagnostic methods must be accurate, simple, and affordable for limiting the spread of infection and for the appropriate clinical management of persons infected with HIV (24). It is imperative that these new diagnostics, like all the other tests, are rigorously and properly evaluated in the situations in which they will be deployed in disease control before they are released for general use. Although fourth-generation HIV tests offer the great advantages of improved precision, reliability, technical simplicity, short turn-around time, high-speed throughput, and full

automation, particularly for high-volume hospital laboratories, they are primarily intended for advanced diagnostic laboratories, and may not address testing methods or strategies in more resource-limited settings, which may not afford the higher reagents cost and cannot explain the higher FPR of the new assay properly. Current HIV test performance could be improved by better standardization of test procedures and institution of mandatory proficiency testing and licensure of clinical laboratories that perform HIV testing, and they should be evaluated in a manner that minimizes referral bias and inadequate reference standard confirmation, problems that have affected the evaluation of current tests. If performance is similar, other characteristics, such as convenience, time to result, shelf life, and cost will likely be the determining factors for selection of a HIV screening test for a specific application.

HIV infection is becoming a chronic disease in increasing numbers of survivors, requiring organized medicine more than ever but limited medical resources may still remain a barrier of effective health care. Though national and provincial level support has been provided to build local capacity in areas affected by HIV, many key challenges and disputes remain in promoting universal access to HIV diagnosis, treatment and care, and support (25, 26). If widespread use of routine screening could offer benefits at a reasonable cost, the potential cost savings are remarkable. However, in developing countries, such as China, it was hard for a laboratory to afford the burden for so many false-positive samples. The mean COI ratios are shown in Table 3, which indicated that the gaps among different HIV infectious states were big. PPVs of two assays (78.35, 82.21%) were not satisfactory in this setting (Table 1). However, in cases with values >50 for the Elecsys[®] HIV combi PT assay and the Elecsys[®] HIV combi assay, nearly all repeated reactive samples were true HIV positive. Over 94% cases with COI ratio <15.0 were false-positive, reflecting the high FPR in weakly reactive samples. To some degree, lots of supplemental test, which is expensive and time-consuming, can be avoided by improving these screening tests.

This study also has limitations. As this is a large retrospectively research, the same specimens were not tested with both assays over the different time periods and lots of specimens were excluded as a result of absencing of follow-up tests results. The cause of absencing of follow-up tests results included the death of the patient, losing contact, and unwillingness. This might influence the results of our evaluation.

In our research, the Elecsys[®] HIV combi PT assay shows a better performance in specificity than the Elecsys[®] HIV combi assay. However, over 94% cases with COI ratio <15.0 were false-positive, this means this screening test still need to be improved and it will need

laboratory personnel to communicate with the clinical doctor and patients more properly about the result of the assay.

ACKNOWLEDGMENTS

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of any organization. The use of trade names is for identification only and does not imply endorsement. The present study was supported by the National Natural Science Foundation of China (81072432), and the Science and Technology Support Program of Sichuan Province (2012FZ0126). Other colleagues who took part in the routine detection but do not become authors also were acknowledged.

REFERENCES

1. Brenner BG, Roger M, Routy JP, et al. High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis* 2007;195(7):951–959.
2. Janssen RS. Implementing HIV screening. *Clin Infect Dis* 2007;45(Suppl 4):S226–31.
3. Miedouge M, Grèze M, Bailly A, Izopet J. Analytical sensitivity of four HIV combined antigen/antibody assays using the p24 who standard. *J Clin Virol* 2011;50(1):57–60.
4. Chinese Center for Disease Control and Prevention. National Guideline for Detection of HIV/AIDS, 2009 edition; 2009, Beijing, China: Chinese Center for Disease Control and Prevention.
5. Mühlbacher A, Schennach H, van Helden J, et al. Performance evaluation of a new fourth-generation HIV combination antigen-antibody assay. *Med Microbiol Immunol* 2013;202(1):77–86.
6. Song EY, Hur M, Roh EY, Park MH, Moon HW, Yun YM. Performances of four fourth-generation human immunodeficiency virus-1 screening assays. *J Med Virol* 2012;84(12):1884–1888.
7. Pasquier C, Sandres-Sauné K, Mansuy JM, et al. Virological exploration of individuals with discordant HIV screening tests. *J Clin Virol* 2004;30(3):218–223.
8. Seo YS, Jung ES, Kim JH, et al. Significance of anti-HCV signal-to-cutoff ratio in predicting hepatitis C viremia. *Korean J Intern Med* 2009;24(4):302–308.
9. O'Brien JE. Hepatitis B surface antigen: Decreased need for confirmation of reactive results. *Clin Chem* 2000;46(4):582.
10. Xu W, Li Y, Wang M, Gu J. Comparison of two immunoassays for determining hepatitis B virus serum markers. *Clin Chem Lab Med* 2011;50(1):153–157.
11. Dufour DR. Hepatitis B surface antigen (HBsAg) assays-are they good enough for their current uses? *Clin Chem* 2006;52(8):1457–1459.
12. Kiely P, Walker K, Parker S, Cheng A. Analysis of sample-to-cutoff ratios on chemiluminescent immunoassays used for blood donor screening highlights the need for serologic confirmatory testing. *Transfusion* 2010;50(6):1344–1351.
13. Kim S, Lee JH, Choi JY, Kim JM, Kim HS. False-positive rate of a “fourth-generation” HIV antigen/antibody combination assay in an area of low HIV prevalence. *Clin Vaccine Immunol* 2010;17(10):1642–1644.
14. García T, Tormo N, Gimeno C, de Lomas JG, Navarro D. Performance of an automated human immunodeficiency virus (HIV)

- antigen/antibody combined assay for prenatal screening for HIV infection in pregnant women. *J Med Microbiol* 2009;58(Pt 11):1529–1530.
15. Weber B. HIV seroconversion: Performance of combined antigen/antibody assays. *AIDS* 2003;17(6):931–933.
 16. Chavez P, Wesolowski L, Patel P, Delaney K, Owen SM. Evaluation of the performance of the Abbott ARCHITECT HIV Ag/Ab Combo Assay. *J Clin Virol* 2011;52(Suppl 1):S51–55.
 17. Masciotra S, McDougal JS, Feldman J, Sprinkle P, Wesolowski L, Owen SM. Evaluation of an alternative HIV diagnostic algorithm using specimens from seroconversion panels and persons with established HIV infections. *J Clin Virol* 2011;52(Suppl 1):S17–22.
 18. Kagulire SC, Stamper PD, Opendi P, Nakavuma JL, Mills LA, Makumbi F, et al. Performance of two commercial immunochromatographic assays for rapid detection of antibodies specific to human immunodeficiency virus types 1 and 2 in serum and urine samples in a rural community-based research setting (Rakai, Uganda). *Clin Vaccine Immunol* 2007;14(6):738–740.
 19. Willman JH, Hill HR, Martins TB, Jaskowski TD, Ashwood ER, Litwin CM. Multiplex analysis of heterophil antibodies in patients with indeterminate HIV immunoassay results. *Am J Clin Pathol* 2001;115(5):764–769.
 20. Zeh C, Oyaro B, Vandenhoude H, et al. Performance of six commercial enzyme immunoassays and two alternative HIV-testing algorithms for the diagnosis of HIV-1 infection in Kisumu, Western Kenya. *J Virol Methods* 2011;176(1–2):24–31.
 21. Jarvis LM, Dow BC, Cleland A, et al. Detection of HCV and HIV-1 antibody negative infections in Scottish and Northern Ireland blood donations by nucleic acid amplification testing. *Vox Sang* 2005;89(3):128–134.
 22. Goodnough LT, Shander A, Brecher ME. Transfusion medicine: Looking to the future. *Lancet* 2003;361(9352):161–169.
 23. Ly TD, Edlinger C, Vabret A. Contribution of combined detection assays of p24 antigen and anti-human immunodeficiency virus (HIV) antibodies in diagnosis of primary HIV infection by routine testing. *J Clin Microbiol* 2000;38(6):2459–2461.
 24. TDR Diagnostics Evaluation Expert Panel, Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, et al. Evaluation of diagnostic tests for infectious diseases: General principles. *Nat Rev Microbiol* 2008;6(11 Suppl):S16–26.
 25. Zhao Y, Poundstone KE, Montaner J, Wu ZY. New policies and strategies to tackle HIV/AIDS in China. *Chin Med J (Engl)* 2012;125(7):1331–1337.
 26. Lai D, Hwang LY, Beasley RP. Compulsory HIV testing for exit from and entry to China: Effectiveness, cost, and stigma—a policy update. *J Public Health Policy* 2012;33(2):269–272.