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Evaluation of the clinical effectiveness of HIV antigen/antibody screening using a chemiluminescence microparticle immunoassay

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Human immunodeficiency virus (HIV) screening assays have improved from single-antigen detection to detection of antigen–antibody combinations. However, concerns have been raised over the potential for false-positive results in antigen–antibody combination assays. The present study investigated the clinical effectiveness of HIV antigen/antibody (HIV Ag/Ab) combination screening by chemiluminescence microparticle immunoassay (CMIA) in over 88,000 samples from an HIV low-prevalence area of Beijing, China. The HIV Ag/Ab CMIA screening results were consistent with those obtained by Western blot and HIV-RNA testing, and had an accuracy of 99.74% (Kappa index = 0.98). False-positive results were more common for women affected by clinical interfering factors (e.g., kidney disease, tumors) than for men (80.95% vs. 15.09%, $P < 0.001$). When CMIA signal-to-cutoff ratio (S/CO) was 11.26, the sensitivity and specificity were highest (100%, 99.43%), and the area under the ROC curve (AUC) was 0.998. Specimens that were negative by CMIA (S/CO <1) were all negative by HIV-RNA testing. These results indicate that HIV Ag/Ab CMIA has a good clinical performance; however, some clinical interfering factors should be considered in HIV low-prevalence areas for their potential to skew testing results.

1. Introduction

The performance of HIV screening assays has improved continuously since the first HIV test was introduced in 1985. Current efforts in screening development focus on tests with a higher accuracy for earlier detection. Individuals with acute infection have a high viral load, which promotes the spread of disease; thus, diagnosis in the early acute period is critical for helping control the epidemic (Pilcher et al., 2001, 2002). After the detection limit of the p24 antigen enzyme immunoassay (EIA) was improved to match that of the single-antigen EIA, HIV antigen/antibody (Ag/Ab) combination assays were introduced and have been implemented worldwide (Weber et al., 2002a,b). These HIV Ag/Ab combination assays have shortened the window compared to those of previous antibody-alone EIAs (Mylonakis et al., 2000; Ly et al., 2004; Patel et al., 2010; Chavez et al., 2011). Many attempts to diagnose acutely infected individuals have applied RNA detection algorithms to pooled HIV antibody-negative specimens. Such efforts have yielded significant returns in detection of recent HIV infection in certain communities (Patel et al., 2006; Fiscus et al., 2007; Priddy et al., 2007). However, the use of RNA-based detection methods, especially during early stages of infection, may lead to more false-negative results (Cohen et al., 2010), and thus is not ideal from an HIV-prevention perspective. In contrast, greater accuracy may be offered through HIV Ag/Ab combination assays, which can detect p24 antigen that appears in the early phase. Because p24 antigen is more stable than HIV RNA and p24 antigen is not like HIV RNA which can be degraded by salivary enzyme in the air, patients with lower concentrations of p24 antigen can be tested by changing the assay conditions. Also, HIV Ag/Ab combination assays are easy to perform, relatively inexpensive, and easily automated.

One such combination assay is the Abbott Architect HIV Ag/Ab Combo, a chemiluminescent microparticle immunoassay (CMIA) run on an automated random-access instrument; it can screen for the HIV antigen/antibody rapidly. Although this HIV Combo can be effective in identifying the maximum possible number of HIV-infected people from a public health perspective, there are concerns about false-positive results (Guinn, 2007; Zdeb, 2007). In addition, the rate of false-positive results may be significantly higher in situations of extremely low HIV prevalence (Guinn, 2007; Shima-Sano et al., 2010). In this study, the clinical performance of the HIV Ag/Ab Combo CMIA was evaluated in a low-HIV prevalence area in Beijing,

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China. The clinical factors interfering with CMIA detection of the HIV antigen/antibody (signal-to-cutoff >1, but negative by Western blotting or HIV-RNA testing) leading to false-positive results were also explored.

2. Materials and methods

2.1. Clinical samples and screening systems

Between May 2012 and June 2013, a total of 88,604 samples (male/female: 37,803/50,801, age: 6 months – 96 years old) were tested for HIV using the HIV Ag/Ab Combo assay (Abbott, Wiesbaden, Germany) at the Peking University First Hospital, China. The HIV Combo is a chemiluminescent magnetic microparticle-based immunoassay and was run on the automated random-access instrument (Abbott, i2000SR). Specimens with signal-to-cutoff (S/CO) ratios of 1.0 or greater were considered reactive. Specimens that tested initially as reactive by CMIA were retested in duplicate using the fourth-generation HIV Ag/Ab ELISA (Merieux, France). All specimens obtained from peripheral blood were stored in aliquots at −70 °C for later testing, and only one freeze/thaw cycle was permitted per aliquot.

The study was approved by the Research and Ethical committees of Peking University First Hospital.

2.2. Gold standard

Specimens were considered to be from HIV-infected individuals if they were repeatedly reactive by ELISA/CMIA and reactive by Western blotting (IMT HIV-1/2 Blot, Shanghai, China) or HIV-RNA testing (Automated nucleic acid detector Procleix Tigris System, San Diego, USA). Specimens with both negative ELISA and negative HIV-RNA results were identified as HIV-negative. Specimens were considered to be from individuals with acute HIV infection if they were sero-negative or indeterminate using ELISA, CMIA, or Western blot, but HIV-RNA-positive.

2.3. Study design

HIV infection status was confirmed with the following algorithm. Specimens with initial HIV-negative results by CMIA were considered uninfected. Any reactive results on a screening test were retested in duplicate. If the secondary test was reactive, the specimen was reported as repeatedly reactive and was submitted for confirmation testing. Confirmatory tests included Western blot and HIV-RNA tests. If the immunoblot result was negative or indeterminate, nucleic acid testing for viral RNA would be suggested to detect an early infection. If the HIV-RNA test was negative, the clinical factors interfering with CMIA detection of the HIV antigen/antibody were considered.

The clinical performance of CMIA was evaluated with 49 specimens from HIV-infected (positive by immunoblot) and 3000 specimens from HIV-uninfected (negative by HIV-RNA). We performed an accuracy study method on the CMIA using a 2×2 contingency table to determine values for positive percent agreement, negative percent agreement, sensitivity, specificity.

A weak-positive specimen and a strong-positive specimen were detected by CMIA (HIV Ag/Ab Combo) for evaluating the precision of this assay. Two batches were tested, and each batch was tested twice daily for 20 days according to the guidelines (EP5-A2) for the evaluation of qualitative assays published by the Clinical Laboratory Standards Institute (CLSI, 2004).

Table 1
The precision of CMIA (HIV Ag/Ab Combo).

Sample	Within-batch		Between-batch	
	SD	CV (%)	SD	CV (%)
Low S/CO	0.1610	3.10	0.2760	5.31
High S/CO	5.9918	2.36	6.1356	2.42

S/CO: signal-to-cutoff, SD: standard deviation, CV: coefficient of variation.

2.4. Statistical analysis

The data were analyzed using SPSS 13.0 (SPSS, Chicago, USA). The receiver–operator curve (ROC) was plotted. Differences of quantitative parameters between two groups were assessed using Mann–Whitney *U* test for data not distributed normally. Pair-wise comparison of independent samples was performed using the χ^2 test. All reported *P* values were two-tailed analyses. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Precision of CMIA (HIV Ag/Ab Combo)

The coefficient of variation (CV) of within and between batch of two concentration samples (low S/CO value of 5.19 ± 0.2865 and high S/CO value of 235.59 ± 6.6397) were calculated according to the guidelines (EP5-A2) for the evaluation of qualitative assays published by the Clinical Laboratory Standards Institute (CLSI). All values for the CV were less than 6% (Table 1).

3.2. Clinical performance of CMIA (HIV Ag/Ab Combo)

Specimens identified as HIV-positive by CMIA were considered to be confirmed positive if they were reactive by immunoblot or HIV-RNA (Kelly et al., 2009; Shima-Sano et al., 2010; CDC, 2013). A total of 49 specimens from HIV-infected individuals (positive by Western blot) and 3000 specimens from HIV-uninfected individuals (negative by HIV-RNA) were detected by CMIA (Table 2). The sensitivity and specificity of CMIA were 100% and 99.93% [99.73%, 99.99%], respectively. Positive percent agreement and negative percent agreement were 96.08% [85.41%, 99.32%] and 100% [99.84%, 100%], respectively. Youden's index was 0.993. CMIA screening results had excellent consistency with the final confirmed results, and the accuracy rate was 99.74% (Kappa index = 0.98, $P < 0.001$).

3.3. Clinical interfering factors affecting CMIA results

There were 81 cases from the 88,604 screening specimens that were S/CO ≥1 by CMIA. The positive rate was 0.091% (81/88,604). The negative (14 cases) and indeterminate (18 cases) specimens by Western blot were validated by HIV-RNA (Table 3).

The ROC curve is shown in Fig. 1. The area under the curve was 0.998 [0.992, 1.003]. When the cutoff value was 11.26, the sensitivity was 100%, and the specificity was 99.43%. At this cutoff, there was only 1 case of interference (S/CO 15.36).

Table 2
Clinical performance of CMIA (HIV Ag/Ab Combo).

		Confirmed results		Total
		Positive	Negative	
CMIA	Positive	49	2	51
	Negative	0	2998	2998
	Total	49	3000	3049

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