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False-Positive Rate of a "Fourth-Generation" HIV Antigen/Antibody Combination Assay in an Area of Low HIV Prevalence▽

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We retrospectively analyzed the performance of the Architect HIV antigen/antibody (Ag/Ab) combination assay in a tertiary health care center with a situation of low HIV prevalence. The specificity and positive predictive value (PPV) were 99.78% and 31.21%, respectively. However, the specificity and PPV could increase to 99.99% and 89.70% using an arbitrary cutoff value.

Since the first HIV test was introduced in 1985, the performances of HIV screening assays have improved continuously. In particular, the HIV antigen/antibody (Ag/Ab) combination assays launched in 1997 have shortened the window period by 4 to 5 days compared to those of the previous antibody-alone enzyme immunoassays (8). After the improvement of the detection limit of the p24 antigen enzyme immunoassay (EIA) to equivalent to that of the single-antigen EIA, HIV Ag/Ab combination assays have been implemented in numerous laboratories throughout the world (7, 9).

The Centers for Disease Control and Prevention (CDC) recommended the expansion of HIV antibody testing from targeted, risk-based testing to universal screening of all adults aged 13 to 64 years in health care settings (2). Although the revised recommendation could be effective in identifying the maximum possible number of HIV-infected people from a public health perspective, there are concerns about false-positive results (3, 11). False-positive HIV screening results could cause substantial psychological distress while waiting for a confirmatory test (10). Guinn estimated the positive predictive value (PPV) of rapid HIV testing in Oregon with 100% sensitivity and 99.9% specificity to be 29% (3). The rate of false-positive results could be dramatically increased in situations of extremely low HIV prevalence. Shima-Sano et al. reported that the PPV of HIV screening results in pregnant women is only 3.7% (5).

According to a report by the Korea Centers for Disease Control and Prevention, 6,120 individuals have been diagnosed with an HIV infection between 1985 and 2008 in Korea (4). Although the number of newly diagnosed HIV infections has increased, the cumulative number of HIV-infected individuals and prevalence were lower than those in other countries. There has been limited study on the rate of false-positive results in HIV screening tests using an automated HIV Ag/Ab combination assay. In the present study, we retrospectively analyzed the performance of an automated HIV Ag/Ab combination assay in a tertiary health care center with a situation of low HIV prevalence.

TABLE 1. Specificities and positive predictive values of the Architect HIV Ag/Ab combination assay[a]

Parameter	Value(s) for assay group		
	Male	Female	Total
Total no. tested	77,708	77,631	155,339
No. of positive initial screening test results (%)	342 (0.440)	201 (0.259)	543 (0.350)
No. of false-positive results (%)	153 (0.197)	193 (0.249)	346 (0.223)
No. of indeterminate results (%)	4 (0.005)	0 (0.000)	4 (0.003)
No. of true HIV-positive results (%)	152 (0.196)	5 (0.006)	157 (0.101)
No. of previously confirmed HIV-positive results (%)	33 (0.042)	3 (0.004)	36 (0.023)
Specificity (%)	99.80	99.75	99.78
95% confidence interval (%)	99.77–99.83	99.71–99.79	99.75–99.80
Positive predictive value (%)	49.84	2.53	31.21
95% confidence interval (%)	44.09–55.59	0.83–5.80	27.22–35.49

[a] Tests with indeterminate results and previously HIV-confirmed samples were excluded in statistical analysis.

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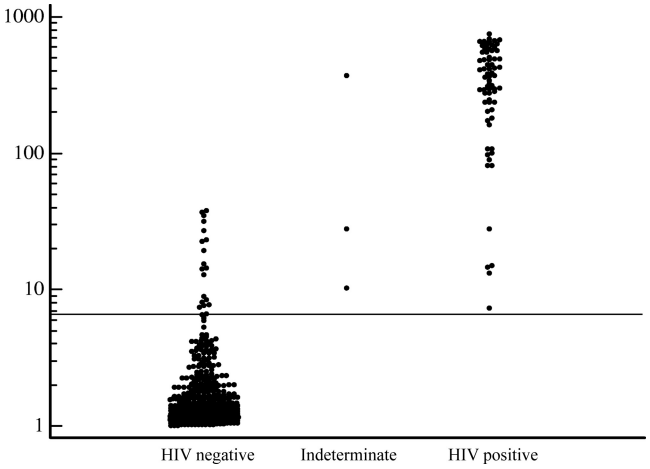

FIG. 1. Distribution of the S/CO ratios of HIV-positive screening samples, according to the results obtained by Western blotting.

During the period of 1 January 2006 through 31 December 2009, a total of 155,339 samples were tested for HIV using Architect HIV Ag/Ab Combo (HIV Combo; Abbott Laboratories, Abbott Park, IL) in a university hospital in South Korea. The Architect HIV Combo test was performed with an automated random access instrument (Architect i2000; Abbott Laboratories) throughout the study period. HIV Combo is a microparticle-based chemiluminescent immunoassay, designed for the simultaneous detection of HIV p24 antigen and HIV-1 and HIV-2 antibodies. Assay results were presented as ratios of specimen signals to the cutoff values (S/CO), where an S/CO ratio greater than or equal to 1.00 is considered reactive. The assays were performed according to the manufacturer's directions.

Specimens that initially tested reactive were retested in duplicate, and repeatedly reactive specimens were subjected to a secondary screening test and confirmatory test. The Vitros anti-HIV 1+2 assay on the Vitros ECiQ immunodiagnostic system (Ortho Clinical Diagnostics, Raritan, NJ) was used as a secondary screening test. Due to the low sample volume, secondary screening assays were performed with 403 specimens among 507 repeatedly reactive specimens. Repeatedly reactive specimens were confirmed by Western blotting (WB) (HIV Blot version 2.2; Genelabs Diagnostics, Singapore) at the Korean National Institute of Health, Seoul, South Korea. Statistical analysis was performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL). This study was approved by the institutional review board of Yonsei University Health System.

A total of 155,339 HIV screening tests for 132,934 patients were performed during the past 4 years; the median patient age was 42.6 years (range, 0 months to 97 years). As shown in Table 1, 543 (0.350%) specimens were found to be reactive using the initial HIV Combo screening assay and repeatedly reactive using the duplicated retesting. By review of the previous laboratory results, 36 specimens were collected from previously confirmed HIV-infected individuals and excluded from supplementary testing such as secondary screening testing or WB analysis. The HIV antibody was confirmed in 157 specimens by WB, and 346 specimens were concluded to have false-positive results, corresponding to a specificity of 99.78% (exact binomial 95% confidence interval [CI], 99.75% to 99.80%). However, PPVs were significantly different between genders, as follows: 49.84% (95% CI, 44.09% to 55.59%) for males and 2.53% (95% CI, 0.83% to 5.80%) for females. These results could be explained by the epidemiologic characteristics of HIV infection in South Korea, showing that approximately 92% of HIV-infected individuals were male.

The mean S/CO ratio of false-positive results was 2.94 (range, 1.00 to 34.59) and that of HIV-positive specimens by WB was 385.97 (range, 7.28 to 739.98) (Fig. 1). The optimal cutoff value based on the highest sum of sensitivity and specificity was estimated to be 8.8, but assay sensitivity was lowered to 99.36% using this cutoff value. With the sensitivity adhering to 100%, the optimal cutoff was revised to 6.6. Using this cutoff value, the assay specificity and PPV were increased to 99.99% (95% CI, 99.98% to 99.99%) and 89.70% (95% CI, 84.00% to 93.62%), respectively (Table 2).

By simply combining the results from the secondary screening test with those from the initial screening test (cutoff, 1.0), the specificity and PPV were increased to 99.99% (95% CI, 99.992% to 99.999%) and 94.44% (95% CI, 85.65% to 98.21%), respectively. Furthermore, by combining the results from the secondary test with those from the initial screening

TABLE 2. Performance of the Architect HIV Ag/Ab combination assay according to cutoff S/CO ratios

Parameter	Value(s) based on cutoff S/CO ratio		
Value of cutoff S/CO ratio	1.0	6.6	8.8
No. of positive initial screening test results (%)	543 (0.350)	216 (0.139)	209 (0.135)
No. of false-positive results (%)	346 (0.223)	19 (0.012)	13 (0.012)
No. of indeterminate results (%)	4 (0.003)	4 (0.003)	4 (0.003)
No. of true HIV-positive results (%)	157 (0.101)	157 (0.101)	156 (0.100)
No. of previously confirmed HIV-positive results (%)	36 (0.023)	36 (0.023)	36 (0.023)
Specificity (%)	100.00	100.00	99.36
95% confidence interval (%)	97.02–100.00	97.02–100.00	95.97–99.97
Positive predictive value (%)	99.78	99.99	99.99
95% confidence interval (%)	99.75–99.80	99.98–99.99	99.99–99.99
No. of true HIV-positive results (%)	31.21	89.70	92.31
No. of previously confirmed HIV results (%)	27.22–35.49	84.00–93.62	86.93–95.67

test using the revised cutoff value of 6.6, there were no false-positive results.

With numerous technical improvements, the current serological HIV screening assays were very sensitive compared to the early immunoassay detecting the HIV antibody. 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. Assay specificity is another important point to consider, and Weber determined the specificity of fourth-generation HIV assays launched after 2001 to be between 99.50% and 99.90% (6). Our study using the Architect HIV Combo assay showed comparable results (specificity, 99.78%; 95% CI, 99.75% to 99.80%).

Even with higher specificity, a low PPV (31.21%; 95% CI, 27.22% to 35.49%) is problematic in situations of low HIV prevalence. In our study, the PPV for males (49.84%; 95% CI, 44.09% to 55.59%) was significantly higher than that for females (2.53%; 95% CI, 0.83% to 5.80%), reflecting the difference of disease prevalence between genders. If 1 million specimens from each gender under the same conditions of prevalence are tested, approximately 1,970 and 2,490 results will be reported falsely positive from males and females, respectively. As the CDC recommended supplemental testing algorithms of antibody to hepatitis C virus for S/CO ratios (1), we could improve the PPV of the HIV Combo assay from 33.21% to 89.20% by setting up an arbitrary revised cutoff S/CO ratio of 6.6. This means that the number of false-positive results will reduce from 346 to only 19 without any further testing. If the secondary screening test is incorporated, the number of false-positive results could be significantly reduced.

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