



Cross-Platform Testing to Reduce False-Positive Results in Fourth-Generation HIV Ag/Ab Assays

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BACKGROUND

- Fourth-generation HIV antigen/antibody (Ag/Ab) assays detect HIV-1 p24 antigen and antibodies to HIV-1/2 simultaneously, enabling earlier diagnosis than previous assays.
- In low-prevalence settings such as Korea, high sensitivity of these assays may increase false-positive results.
- We evaluated whether cross-platform testing with different fourth-generation HIV Ag/Ab assays could reduce false positive results.
- In addition, we investigated whether signal-to-cutoff (S/CO) values generated by these assays could predict final diagnostic outcomes.

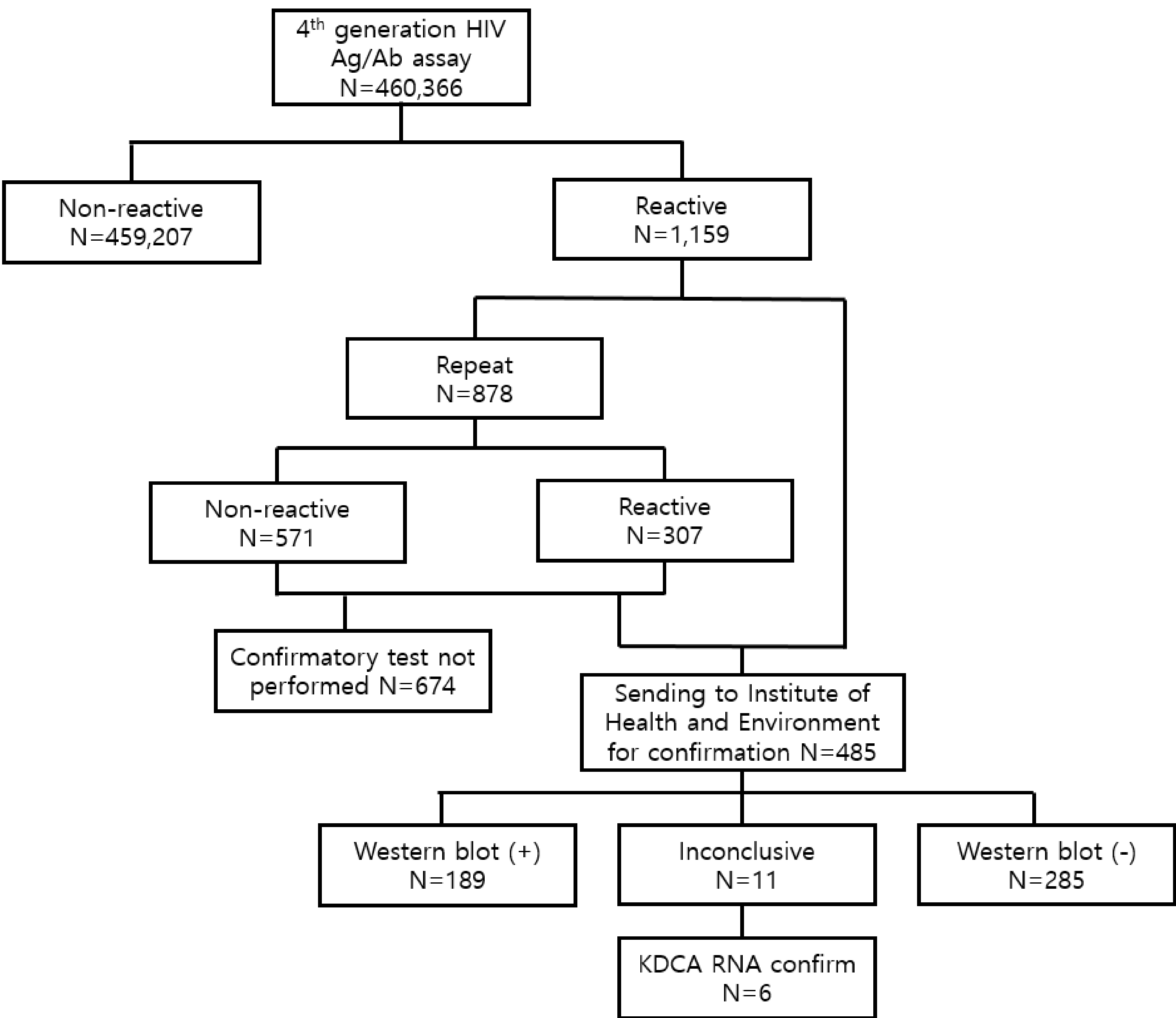
MATERIALS & METHODS

- We reviewed HIV Ag/Ab tests performed at three laboratories from August 2019 to December 2024 using Cobas e801, Alinity i, and ADVIA Centaur XP platforms.
- Samples with borderline (S/CO 0.9–0.99) or positive (S/CO ≥1) results were considered reactive.
- Reactive samples underwent repeat testing using the same or different platforms (cross-platform testing), or were referred for confirmatory Western blot testing. HIV RNA testing was performed at Korea Disease Control and Prevention Agency (KDCA) for inconclusive Western blot results.

RESULTS

- Among 460,366 HIV Ag/Ab tests performed, 1,159 (0.25%) were reactive.

Figure 1. HIV diagnostic algorithm and sample results distribution



- When repeat testing was performed using the same platform, 30 of 303 samples (9.9%) reverted to non-reactive. In contrast, 572 of 724 samples (79.0%) reverted to non-reactive when retested on a different platform.

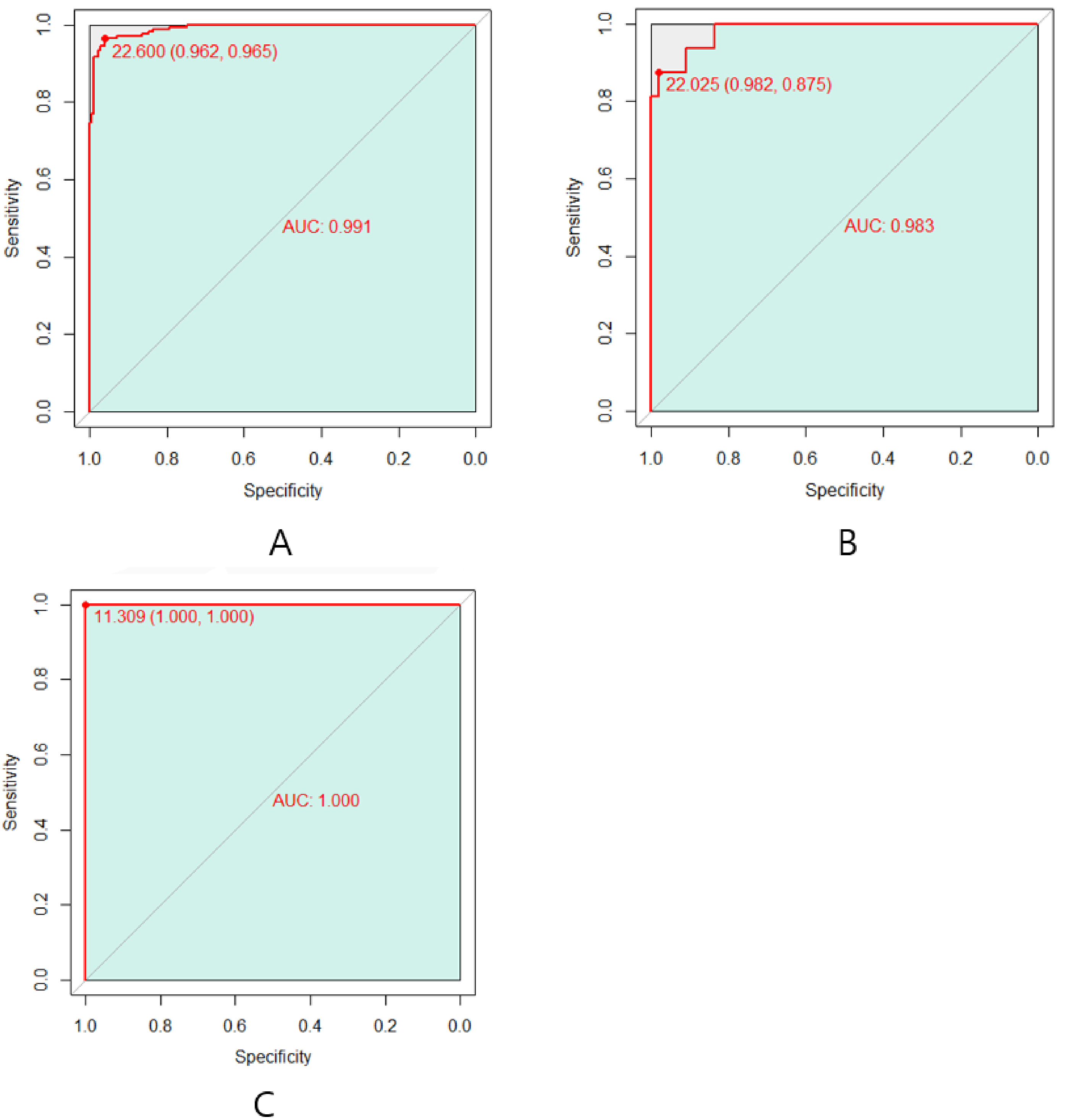
- All 30 samples that reverted to non-reactive on the same platform were also non-reactive on cross-platform testing.
- All 55 specimens that became non-reactive and underwent confirmatory testing were confirmed negative.
- Among 152 samples that remained reactive after cross-platform testing, 34 were confirmed negative and 114 were confirmed positive. 4 samples show inconclusive results.

Table 1. Number of samples that reverted to non-reactive after repeat testing by testing strategy and year. Values are expressed as n/N (%), where n is the number of samples reverted to non-reactive results and N is the total number of samples in each category.

Testing strategy	2019	2020	2021	2022	2023	2024
Total repeat testing	5/24 (20.8%)	19/129 (14.7%)	76/126 (60.3%)	146/191 (76.4%)	170/208 (81.7%)	156/200 (78.0%)
Repeat testing on the same platform	0/18 (0.0%)	4/118 (3.4%)	4/100 (4.0%)	22/57 (38.6%)	0/10 (0.0%)	*
Repeat testing on the different platform	5/9 (55.6%)	19/23 (82.6%)	76/107 (71.0%)	146/177 (82.5%)	170/208 (81.7%)	156/200 (78.0%)

- Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal S/CO cut-off values for predicting Western blot results for each assay platform.

Figure 2. ROC curve for the each platform's S/CO ratio, with the AUC and optimal threshold maximizing sensitivity and specificity. (A) cobas platform (B) Alinity platform (C) ADVIA platform



CONCLUSION

- Cross-platform testing using different fourth-generation HIV Ag/Ab assays substantially reduced false-positive HIV screening results in this low-prevalence setting.
- This approach may help reduce unnecessary confirmatory testing and improve the efficiency of HIV diagnostic workflows in routine clinical laboratories.