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Performance of an Alternative Laboratory-Based HIV Diagnostic Testing Algorithm Using HIV-1 RNA Viral Load

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Abstract

Background: Since 2014, the recommended algorithm for laboratory diagnosis of HIV infection in the United States has consisted of an HIV-1/2 antigen/antibody (Ag/Ab) test followed by an HIV-1/2 antibody (Ab) differentiation test and, if necessary, a diagnostic HIV-1 nucleic acid test (NAT) to resolve discordant or indeterminate results.

Methods: Using stored specimens from persons seeking HIV testing who had not received a previous diagnosis or treatment, we compared the performance of a three-step alternative algorithm consisting of an Ag/Ab test followed by a quantitative HIV-1 RNA viral load assay and, if viral load is not detected, an Ab differentiation test, to that of the recommended algorithm. We calculated the sensitivity and specificity of five Ag/Ab tests and the proportion of specimens correctly classified by the alternative algorithm compared to the recommended algorithm. Results were examined separately for specimens classified as early infection, established infection, and false-reactive screening

Results: Sensitivity and specificity were similar among all Ag/Ab tests. Viral load quantification correctly classified all specimens from early infection, all false-reactive screening specimens, and the majority of specimens from established infection.

Conclusions: Although cost, regulatory barriers, test availability, and the ability to differentiate early from established infection must be considered, this alternative algorithm can potentially decrease the total number of tests performed and reduce turnaround time, thereby streamlining HIV diagnosis and initiation of treatment.

Short Summary

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This evaluation compared the performance of an alternative diagnostic algorithm that uses HIV-1 RNA viral load for confirmation of reactive screening results to the recommended laboratory testing algorithm.

Keywords

HIV testing; algorithm; viral load; sensitivity; specificity

Introduction

In 2014, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) issued updated recommendations for laboratory testing of serum and plasma specimens for diagnosis of HIV infection in the United States (1). The recommendation included an updated diagnostic testing algorithm consisting of an HIV-1/2 antigen/antibody (Ag/Ab) test followed, if reactive, by an HIV-1/2 antibody (Ab) differentiation test and, if necessary to resolve discordant or indeterminate results, an HIV-1 nucleic acid test (NAT). This recommended algorithm has been widely adopted by public health and commercial laboratories across the United States (2,3). By 2015, this algorithm was used to establish the majority of all HIV diagnoses reported to CDC (4). However, some implementation challenges remain. Interpretation of algorithm results has been complicated by the introduction of newer Ag/Ab tests with the ability to differentiate HIV-1 p24 Ag reactivity from Ab reactivity (5,6). Additionally, the advent of a new HIV-1/2 Ab differentiation test that can generate additional test interpretations relative to other supplemental tests (7) might increase the occurrence of ambiguous results, such as HIV-2 indeterminate or HIV indeterminate results, which may require subsequent nucleic acid testing of specimens that are ultimately found to be HIV-negative (3,8). Currently, only one NAT, the Aptima HIV-1 RNA Qualitative Assay (Apt-Qual; Gen-Probe Inc., San Diego, CA), has received Food and Drug Administration (FDA) approval for HIV diagnosis. However, because Apt-Qual testing is resource intensive, many laboratories do not perform these tests and instead send specimens to a central reference laboratory for this testing, leading to potential diagnostic or reporting delays (9). Some studies have suggested that this recommended algorithm might also increase cost per HIV-positive specimen in certain laboratory settings relative to the previous laboratory testing algorithm (10,11).

To address some of these challenges, alternative algorithms have been proposed in which a quantitative rather than qualitative NAT is performed, either as a supplemental test following a reactive Ag/Ab test as evaluated in this study and proposed by others (9,12,13), or as a third test to resolve reactive Ag/Ab and negative supplemental test results (14,15). An alternative algorithm in which a quantitative NAT with a diagnostic indication is the second step in the algorithm could potentially streamline diagnosis and reduce the number of specimens with ambiguous Ab differentiation results. We evaluated the performance of a three-step alternative algorithm consisting of an Ag/Ab test followed by an HIV-1 RNA viral load assay and an Ab differentiation test performed only when HIV-1 viral load is not detected compared to the currently recommended algorithm (Supplemental Figure 1).

Methods

This study examined serum and plasma specimens collected during an earlier evaluation of the performance of HIV rapid tests among persons seeking HIV testing at two clinics in Los Angeles, CA, described elsewhere (16,17). During 2003–2005, 5,789 persons with unknown HIV status provided capillary whole blood, anticoagulated (EDTA) venous whole blood, and serum specimens. These individuals were considered to be antiretroviral treatment (ART) naive based on self-reported unknown HIV status. Remnant serum and plasma specimens were stored at −70℃ for subsequent testing with newer tests. This study evaluated the subset of 1,171 stored specimens that were tested with 5 Ag/Ab tests.

The five Ag/Ab tests examined in this analysis were ARCHITECT HIV Ag/Ab Combo (ARC; Abbott Laboratories, Abbott Park, IL), GS HIV Combo Ag/Ab EIA (GS; Bio-Rad Laboratories, Inc., Redmond, WA), ADVIA Centaur HIV Ag/Ab Combo (CHIV; Siemens, Tarrytown, NY), BioPlex 2200 HIV Ag-Ab (BPC; Bio-Rad Laboratories, Inc., Hercules, CA), and Determine HIV-1/2 Ag/Ab Combo (DC; Abbott Laboratories, Abbott Park, IL). All Ag/Ab tests were performed with serum specimens. ARC and GS tests were performed in 2008, CHIV and DC tests were performed in 2014, and BioPlex tests were performed in 2015. ARC, CHIV, and BioPlex tests were performed at their respective test manufacturer laboratories. Because specimen volume was limited, only a subset of specimens with initially reactive Ag/Ab results were repeated in duplicate as recommended for all Ag/Ab tests but the DC test; therefore, only the initial reactive results were considered in this study.

Ab differentiation testing for both the recommended and alternative algorithms was performed with the Geenius HIV-1/2 Supplemental Assay (Geenius; Bio-Rad Laboratories, Inc., Redmond, WA). Geenius tests were performed in 2014 using version 1.1 of the automated reader software, before the cut-off value of the HIV-2 gp140 band was raised (8). Apt-Qual was used to confirm discordant Ag/Ab and supplemental Ab results as indicated in the recommended algorithm.

In the alternative algorithm evaluated in this study, HIV-1 RNA quantification at the second step was performed with the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 (Roche; Roche Molecular Systems, Inc., Branchburg, NJ) and the Aptima HIV-1 Quant Assay (Apt-Quant; Hologic, Inc., San Diego, CA), which are not currently FDA approved for HIV diagnosis. Both viral load assays were performed in 2016 with plasma specimens using off-label dilution protocols. For Roche tests, 100 μL of plasma were used for a 1:5 dilution protocol with an expected limit of quantification (LOQ) ~ 100 copies/mL. For Apt-Quant tests, 100 μL of plasma were used for a 1:7 dilution protocol with an expected LOQ ~ 210 copies/mL. Because specimen volume was limited, not every specimen was tested with both viral load assays.

We evaluated specimens with results for all 5 Ag/Ab tests. We first calculated the sensitivity, specificity, and corresponding exact 95% confidence intervals (CI) of each of the 5 Ag/Ab tests during early and established infection using the recommended algorithm as the reference standard. For calculation of sensitivity of Ag/Ab tests, specimens with negative or indeterminate Geenius results and reactive Apt-Qual results were classified as early infection

(n=13), regardless of Ag/Ab screening result. Specimens with reactive Ag/Ab results and HIV-1 positive Geenius results were classified as established infection (n=170). Specimens previously determined to be Ab and Apt-Qual negative (16) were classified as HIV-negative (n=988). Next, we examined algorithm outcomes among specimens with a reactive result on at least 1 Ag/Ab test. For each screening test, we calculated the proportion of specimens correctly classified by the first 2 steps of the alternative algorithm (Ag/Ab test followed by viral load assay) relative to the recommended algorithm. Geenius results (i.e., the third step of the alternative algorithm) were examined for specimens with discordant Ag/Ab and viral load results (i.e., initially reactive screening and undetectable viral load). The performance of the alternative algorithm was evaluated separately during early and established infection with specimens with a reactive Ag/Ab result, as well as specimens with a false reactive result on any Ag/Ab test (defined as initially reactive Ag/Ab, HIV negative or indeterminate Geenius, and nonreactive Apt-Qual results). For specimens tested with both Roche and Apt-Quant, viral loads were plotted and Pearson's correlation coefficient was used to assess the concordance of viral loads quantified by the two assays.

Results

Performance characteristics of each of the 5 Ag/Ab tests were similar (Table 1); however, not all testing was performed as recommended by the manufacturer. All Ag/Ab tests showed high sensitivity (≥ 99.4%) during established infection, but sensitivity was lower during early infection and ranged from 31–54%. Sensitivity of DC was lower than that of the 4 instrumented laboratory-based tests, but CIs for each test were wide and overlapping. Specificity was similarly high among all Ag/Ab tests.

Roche was performed with 193 1:5 diluted plasma specimens, which included 38 algorithm-defined false-positive specimens and 155 algorithm-defined positive specimens. Of the 155 algorithm-defined positive specimens, 8 had undetectable viral loads, 2 had detected viral loads below the expected lower LOQ of 100 copies/mL, 1 had viral load above the expected upper LOQ of 10 million copies/mL, and 11 had quantified viral loads <1,000 copies/mL. The remaining 133 (86%) algorithm-defined HIV-positive specimens had quantified viral loads >1,000 copies/mL. Apt-Quant was performed with a subset of 113 algorithm-defined positive specimens. All 113 diluted plasma specimens had quantified viral loads >1,000 copies/mL. Roche and Apt-Quant diluted viral load results were correlated (R^2=0.82) (Supplemental Figure 2) among the 113 specimens with quantifiable results on both tests.

Supplemental Figure 3a and 3b show outcomes of the recommended and alternative algorithms, respectively, among the 193 specimens examined. Of the 155 algorithm-defined positive specimens with a Roche result (either detected or undetectable viral load), 5 were from persons with early infection and 150 from persons with established infection (Table 2). Using the Roche viral load assay as the second step of the alternative algorithm, viral load was detected in 5 (100%) of the specimens collected during early infection; viral load was quantified at >1,000 copies/mL in 4 of these specimens and was above the upper LOQ for 1 specimen. Viral load was detected in 142 (94.7%) of the specimens collected during established infection; all were quantified except 2 that were detected <LOQ. The proportion

of specimens correctly classified by Ag/Ab screening followed by Roche viral load did not vary by individual Ag/Ab test.

Of the 113 specimens tested with Apt-Quant, 4 specimens from persons with early infection and 109 specimens from persons with established infection were initially reactive on one or more Ag/Ab test and had a detectable Apt-Quant viral load. Of the 4 from early infection, 2 viral loads were quantifiable and 2 were detected >LOQ. Of the 109 from established infections, all were quantified at >1,000 copies/mL. Using Apt-Quant as the second step of the algorithm, viral load was detected in all specimens collected during early and established infection, independent of the specific Ag/Ab test used.

All of the 8 algorithm-defined HIV-positive specimens from persons with established infection that had initially reactive Ag/Ab and undetectable Roche viral load were HIV-1 positive by Geenius and therefore classified as HIV-1 positive by the alternative algorithm. Signal-to-cutoff ratios for each Ag/Ab test, as well as Geenius bands present and test interpretations and Apt-Qual results, are displayed in Supplemental Table 1, for each of these 8 specimens. Three of these 8 algorithm-defined HIV-positive specimens were positive by Apt-Qual. One specimen was false-negative by DC; both the alternative and recommended algorithms alone would have failed to identify this infection if DC was the initial test used.

Of the 38 algorithm-defined HIV-negative specimens with an initially false reactive Ag/Ab result; 8 had an initially false reactive result on more than one Ag/Ab test. Ag/Ab test signal-to-cutoff ratios, Geenius bands and test interpretations, and Apt-Qual and Roche viral load results for these specimens are displayed in Supplemental Table 2. All 38 of these specimens were correctly classified by Roche (i.e., undetectable viral load); none were tested with Apt-Quant. Consistent with the Roche result, all of these specimens also had negative results on the FDA-approved Apt-Qual test. Because these specimens had discordant Ag/Ab and Roche viral load results, they would proceed to Ab supplemental tests if the proposed alternative algorithm were followed. Of the 38 specimens, 33 were HIV negative by Geenius, 1 was HIV-1 indeterminate, and 4 were HIV-2 indeterminate; of the 4 HIV-2 indeterminate specimens, 3 underwent repeated Geenius testing, and 2 were found to be HIV negative while 1 remained HIV-2 indeterminate. Three of the 4 initial HIV-2 indeterminate specimens had only the gp140 band detected; band results were not available for the remaining HIV-2 indeterminate specimen. Under the recommended algorithm, all 38 specimens would have proceeded to Geenius followed by Apt-Qual testing, which would have identified all 38 specimens as HIV-1 negative (with potential referral for HIV-2 testing or subsequent follow-up testing of specimens with Geenius indeterminate results (18)).

Discussion

In this evaluation of stored specimens, an alternative algorithm that used an HIV-1 RNA viral load assay as the second step performed well overall, correctly classifying the majority (94.6%) of HIV-1 positive specimens within two steps and all specimens after Geenius results were examined as a third step. The observed performance of this alternative algorithm was consistent with other evaluations (12,19) and robust to the use of different

Ag/Ab tests and viral load assays. There was no meaningful difference in the performance of the instrumented laboratory-based and rapid Ag/Ab test using serum specimens, and algorithm outcomes did not vary by screening test, although only initial screening test results were considered. Likewise, among the specimens tested with both viral load assays, algorithm outcomes did not vary, and concordance between the two viral load assays was high.

A key component of this alternative algorithm is the reduced reliance on the FDA-approved HIV-1 RNA qualitative assay. Of the 193 Ag/Ab-reactive specimens included in our study, 43 (22%) would have required a qualitative NAT to confirm diagnosis using the recommended algorithm. The single commercially available qualitative NAT with a diagnostic claim in the United States is labor intensive and not universally adopted by public health laboratories, particularly those with low overall HIV testing volume or limited resources. In 2017, nearly two thirds of surveyed U.S. public health laboratories referred specimens to another laboratory for qualitative HIV-1 NAT rather than performing these tests in-house (2). Referral can lead to substantial delays in reporting of results, which can negatively affect core public health activities such as case investigation and partner services. Because HIV-1 viral load assays are more commonly available in commercial and hospital laboratories and serve as the necessary next step after diagnosis of an HIV infection, this alternative algorithm could potentially streamline diagnosis and reporting of test results. An algorithm that includes quantitative viral load testing for diagnosis could therefore provide results to confirm diagnosis and reduce the total number of tests performed. FDA approval of NATs with both diagnostic and prognostic (e.g., viral load) claims could lead to improved algorithms for HIV testing.

The described alternative algorithm can also avert Ab differentiation testing for the majority of specimens tested without sacrificing the ability to diagnose HIV-2 infections. In this evaluation, only 46 of the 193 specimens with a reactive Ag/Ab result that were followed through the alternative algorithm (i.e., 38 Ag/Ab false reactive and 8 HIV-positive with undetectable viral load) would have proceeded to Ab differentiation testing as a third step. Of these 46, only 5 would have required further testing beyond the alternative algorithm as a result of Geenius indeterminate results. However, if the recommended algorithm were followed, all 193 of these specimens would have been tested with an Ab differentiation test, increasing the likelihood of encountering ambiguous test results (e.g., HIV-2 indeterminate results as described by Wesolowski et al. in this issue (3)). Diagnosis of HIV-2 infections using the alternative algorithm would require three steps, but would ultimately be confirmed by Ab differentiation as with the recommended algorithm.

Of the 193 Ag/Ab-reactive specimens, a similar number of specimens would have proceeded to the third step of either the alternative or recommended algorithm (46 and 43, respectively). However, the recommended algorithm does not account for viral load assays following diagnosis, while the alternative algorithm includes a viral load assay that could be used for both diagnosis and prognosis, thus decreasing the total number of tests performed. Additionally, viral load assays may be more widely available and therefore allow faster turnaround time than qualitative NAT for diagnosis.

Despite the potential advantages of the alternative algorithm, several complicating factors warrant consideration. The recommended algorithm was designed to facilitate the identification of acute infection and distinguish between acute infection and false-positive Ag/Ab results (1). However, the proposed alternative algorithm might not effectively differentiate between early and established infection since specimens reactive on screening would proceed directly to a viral load assay without establishing whether the patient had a mature antibody response (20). Nevertheless, under an algorithm that provides quantitative viral load as part of diagnosis, high viral load could be used with CD4 count to indicate possible early or late-stage infection and inform public health action to prevent transmission. Alternatively, Ag/Ab tests that provide results separately for each analyte may be able to assist with the identification of early infection, if specimens with detected Ag, but without detected Ab are assumed to represent early infection. Future evaluations will need to explore ways to mitigate the potential clinical and public health impacts of an algorithm that can detect early infections, but might not differentiate early from established infections.

Logistical barriers to algorithm implementation should also be considered. Since HIV-1 RNA viral load assays currently lack FDA approval for diagnosis, these tests cannot be used for that purpose unless a validation study is performed (incurring significant up-front costs) or the test is ordered by a physician (9). In addition to the up-front costs of validation studies, the cost of performing a viral load assay instead of an Ab differentiation test on every specimen with a reactive screening result (including false reactive screening results, which comprised 20% of the Ag/Ab-reactive specimens included our study) may be prohibitive in some laboratory settings. Although any individual diagnosed with the recommended algorithm would ultimately receive a viral load assay before or during treatment initiation, the alternative algorithm might shift the cost of this viral load assay to a different laboratory or agency. Furthermore, viral load assays might be less widely available than first- and second-step serologic assays in some local testing centers, which could potentially delay confirmation of results. Future studies can evaluate the cost of this and other alternative algorithms relative to the recommended algorithm and explore potential reimbursement models that can increase the feasibility of viral load testing as part of diagnosis. FDA approval of viral load assays for HIV-1 diagnosis in the United States could facilitate implementation of the alternative algorithm and reduce cost of the algorithm.

This analysis is subject to limitations. First, off-label dilution protocols were used with both viral load assays to conserve specimen volume. Both protocols had a higher expected LOQ (Roche, 100 copies/mL; Apt-Quant, 210 copies/mL) than the FDA-approved LOQ of these assays (20 copies/mL and 30 copies/mL, respectively). Therefore, sensitivity to detect viral load might have been reduced relative to FDA-approved protocols, which might have contributed to the finding of 8 HIV-positive specimens with undetectable viral load. Second, the tests examined in this analysis were performed on stored specimens collected more than a decade ago, potentially compromising the integrity of test results. Additional evaluations of the alternative algorithm using prospectively collected specimens would be useful. Third, repeated testing of initially reactive specimens was not performed with every specimen or every test for which this practice is recommended by test manufacturers. Therefore, the number of specimens considered to be Ag/Ab false-reactive may represent an overestimate, and specificity of the Ag/Ab screening tests may have been underestimated compared to

tests performed according to package inserts. Fourth, Geenius tests were performed with software version 1.1, prior to the update to raise the cutoff value for the HIV-2 gp140 band (8). We observed four gp140-only HIV-2 indeterminate results, and these results might have been different if the updated software version had been used. Fifth, nearly all detected viral loads were quantified at >1000 copies/mL. Future evaluations should consider the performance of this alternative algorithm with detected but low viral loads, which may be of growing importance in the era of pre-exposure prophylaxis (PrEP) and early treatment. Sixth, the small number of early infections in this study might have hindered comparison of algorithm performance in diagnosis of early infections. Finally, this study did not include HIV-2 positive specimens, so the performance of the alternative algorithm with respect to HIV-2 diagnosis could not be empirically evaluated.

Conclusion

This alternative laboratory testing algorithm was able to correctly identify all specimens tested, including false-positive specimens and those collected during early infection. Regulatory barriers, cost, availability of viral load assays, and the ability to differentiate early from established infection could pose challenges to widespread implementation of the algorithm. Nevertheless, if these barriers are addressed, the algorithm has the potential to decrease the total number of tests performed and reduce turnaround time for indeterminant serologic reactivity, thereby streamlining HIV diagnosis and initiation of treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Sensitivity and Specificity of 5 Ag/Ab Tests Using 1,171 Stored Serum and Plasma Specimens Collected from Persons Seeking HIV Testing in Los Angeles, CA, 2003–2005.

	Early Infection* N=13		Established Infection† N=170		Uninfected‡ N=988	
Ag/Ab Test	**Reactive (n)**	**Sensitivity (95% CI)**	**Reactive (n)**	**Sensitivity (95% CI)**	**Nonreactive (n)**	**Specificity (95% CI)**
Abbott ARCHITECT	7	54% (25%-81%)	170	100% (97.9%-100%)	971	98.3% (97.3%-99.0%)
Bio-Rad GS Combo	7	54% (25%-81%)	170	100% (97.9%-100%)	977	98.9% (98.0%-99.4%)
Siemens ADVIA Centaur	7	54% (25%-81%)	170	100% (97.9%-100%)	977	98.9% (98.0%-99.4%)
Bio-Rad BioPlex 2200	7	54% (25%-81%)	170	100% (97.9%-100%)	974	98.6% (97.6%-99.2%)
Alere Determine	4	31% (9.1%-61%)	169	99.4% (96.8%-100%)	978	99.0% (98.2%-99.5%)

Abbreviations: HIV, human immunodeficiency virus; Ag, antigen; Ab, antibody; CI, confidence interval.

Note: because not all tests with initially reactive Ag/Ab results were repeated in duplicate as recommended by manufacturers, only the initial reactive results were considered in this study.

*defined as negative or indeterminate Geenius and reactive Aptima HIV-1 RNA Qualitative Assay regardless of Ag/Ab screening result

†defined as reactive Ag/Ab and HIV-1 positive Geenius

‡defined based on previously reported (16) nonreactive Ab and Aptima HIV-1 RNA Qualitative Assay

Table 2.

Proportion of Specimens Correctly Classified by Ag/Ab Screening Followed by Viral Load Quantification Using 5 Ag/Ab Tests and 2 HIV-1 Viral Load Assays.

	Roche			Apt-Quant*	
Ag/Ab Test	**Early infection† (n correct/total)**	**Established infection‡ (n correct/total)**	**False reactive§ (n correct/total)**	**Early infection† (n correct/total)**	**Established infection‡ (n correct/total)**
Abbott ARCHITECT	5/5	142/150	13/13	4/4	109/109
Bio-Rad GS Combo	5/5	142/150	11/11	4/4	109/109
Siemens ADVIA Centaur	5/5	142/150	11/11	4/4	109/109
Bio-Rad BioPlex 2200	5/5	142/150	13/13	4/4	109/109
Alere Determine	4/4	142/149	3/3	3/3	109/109
Total (any test)	5/5	142/150	38/38	4/4	109/109

Abbreviations: HIV, human immunodeficiency virus; Ag, antigen; Ab, antibody; Roche, Roche COBAS AmpliPrep/ COBAS TaqMan HIV-1 Test; Apt-Quant, Hologic Aptima HIV-1 Quant Assay.

Note: These specimens represent the subset of specimens tested with all 5 Ag/Ab tests, reactive on at least 1 Ag/Ab test, and tested with at least 1 viral load assay. Two such specimens collected during early infection and 20 specimens collected during established infection were not tested with a viral load assay and are therefore not included in this Table. Because not all tests with initially reactive Ag/Ab results were repeated in duplicate as recommended by manufacturers, only the initial reactive results were considered in this study.

*Specimens with false reactive screening results were not tested with the Hologic Aptima HIV-1 Quant Assay.

†defined as reactive Ag/Ab, negative or indeterminate Geenius, and reactive Aptima HIV-1 RNA Qualitative Assay

‡defined as reactive Ag/Ab and HIV-1 positive Geenius

§defined as reactive Ag/Ab, negative or indeterminate Geenius, and nonreactive Aptima HIV-1 RNA Qualitative Assay