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Alternative Algorithms for Human Immunodeficiency Virus Infection Diagnosis Using Tests That Are Licensed in the United States[▽]

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Serodiagnosis of human immunodeficiency virus (HIV) infection in the United States has traditionally relied on a sequential two-test algorithm: an initial screen with an enzyme immunoassay (EIA) and reflex testing of EIA-reactive specimens with a more specific supplemental test such as Western blotting or immunofluorescence. The supplemental tests are tedious, subjective, and expensive. In addition, there have been major improvements in the performance and accuracy of the EIA tests as well as the introduction of rapid serologic tests (RT) and HIV nucleic acid amplification tests (NAAT). Related to these improvements is the possibility that alternative algorithms using combinations of currently approved HIV tests may function as well as if not better than the current algorithm, with more flexibility, improved accuracy, and lower cost. To this end, we evaluated the performance of 12 currently licensed tests and 1 in-house HIV test (6 EIA, 4 RT, and 3 NAAT) on panels of plasma samples from HIV-infected (*n* = 621 HIV type 1 [HIV-1] and 34 HIV-2) and uninfected (*n* = 513) people and of sequential specimens from people early in seroconversion (183 specimens from 15 patients). Test combinations were analyzed in two dual-test (sensitivity-optimized and specificity-optimized) algorithms and in a three-test (tie-breaking) algorithm, and performance was compared to the conventional algorithm. The results indicate that alternative algorithm strategies with currently licensed tests compare favorably with the conventional algorithm in detecting and confirming established HIV infection. Furthermore, there was a lower frequency of discordant or indeterminate results that require follow-up testing, and there was improved detection of early infection.

Tests for the diagnosis of human immunodeficiency virus (HIV) infection that are currently approved by the U.S. Food and Drug Administration (FDA) have high sensitivity and specificity, exceeding 98% in most cases. In principle, tests with high sensitivity (percent positive in those with infection) are used for screening, with the aim of detecting the largest possible number of specimens from those with true infection at the expense of incorrectly classifying some specimens from uninfected persons as false positive. Thus, a negative test result with a highly sensitive screening test is most useful for ruling out infection, but some positive test results will be incorrect. Conversely, tests with high specificity (percent negative in those without infection) are useful for diagnosing infection when the test result is positive, but some negative test results can be incorrect. In practice, HIV screening and diagnosis involves a testing sequence or algorithm using two or more tests. The strategy of the algorithm is to capture all true positives and a few false positives with a highly sensitive screening test and resolve positive specimens with a more specific test for confirmation. Optimally designed, this leaves, hopefully, only a small number of discordant specimens (screening test positive/confirmatory test negative) that need further testing or follow-up specimens for resolution of infection status.

The U.S. Public Health Service (PHS)-recommended algorithm is a two-test sequence. Specimens are screened with an enzyme immunoassay (EIA), and repeatedly reactive specimens are subjected to supplementary testing with Western blotting or with an immunofluorescence assay (6, 24, 27). The algorithm has been the diagnostic standard in the United States for almost 2 decades. However, the supplementary tests are subjective, expensive, labor-intensive, and subject to shortages. Over the past decade, EIA tests have evolved considerably based on improvements in the target HIV antigens and assay formats. First-generation EIAs detected antibody bound to solid-phase viral lysate. Second-generation EIAs detect antibody to recombinant viral proteins or peptides that are used in place of or in addition to viral lysate. Third-generation EIAs detect antibody using an antigen-antibody-antigen sandwich technique. Fourth-generation EIAs, none of which are currently FDA approved, combine detection of HIV antibody with detection of HIV antigen. These refinements have resulted in improved sensitivity and specificity and more-comprehensive detection of HIV subtypes, groups, and antibody isotypes. Furthermore, these EIAs often detect recent infection earlier than Western blotting (1, 6, 18, 24, 26, 31, 39). Algorithms using only EIAs have been used extensively in international settings with satisfactory results (6, 14). In addition, there has been an expansion in suitable specimen types (saliva, whole-blood finger stick), increasing the options for testing programs (6, 24, 31). Simple, rapid tests have become available that enable testing at the point of client contact in outreach settings outside the laboratory, and an algorithm

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composed exclusively of tests that can be performed on site while the client waits would be highly desirable (6, 14, 15, 24, 38). Nucleic acid amplification tests (NAAT) have been used to identify primary HIV infection before seroconversion and may have an appropriate and useful role in screening and diagnostic algorithms (2, 6, 26, 31, 33, 35, 36, 36, 37, 39, 41).

Because of all these developments and recognition that separate algorithms may be adequate for different purposes, we evaluated the performance of FDA-approved tests in the context of multiple diagnostic algorithms.

MATERIALS AND METHODS

HIV assays. The names, abbreviations, and sources of the HIV assays used in this study are as follows (Table 1): Genetic Systems HIV-1/HIV-2 Plus O EIA (GS HIV-1/2+O; Bio-Rad Laboratories, Redmond, WA); Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA (referred to herein as "Abbott"; Abbott Laboratories, Abbott Park, IL); Vironostika HIV-1 Plus O Microelisa system (Vir HIV-1+O; BioMerieux Inc., Durham, NC); Genetic Systems HIV-1/HIV-2 peptide EIA (GS HIV-1/2 peptide; Bio-Rad Laboratories, Redmond, WA); Genetic Systems rLAV EIA (GS rLAV; Bio-Rad Laboratories, Redmond, WA); Vironostika HIV-1 Microelisa system (Vir HIV-1; BioMerieux Inc., Durham, NC); OraQuick rapid HIV-1/2 antibody test (Oraquick; OraSure Technologies, Inc., Bethlehem, PA); Reveal rapid HIV-1 antibody test (Reveal; MedMira Laboratories, Inc., Halifax, Nova Scotia, Canada); Multispot HIV-1/HIV-2 rapid test (Multispot; Bio-Rad Laboratories, Redmond, WA); Uni-Gold recombigen HIV (Uni-Gold; Trinity Biotech USA, St. Louis, MO); Procleix HIV-1 discriminatory assay (Procleix; now packaged and approved for acute HIV-1 diagnosis as the Aptima HIV-1 qualitative assay; Gen-Probe, Inc, San Diego, CA); CDC in-house assay for HIV-1 RNA (CDC RNA; reference 29); COBAS AmpliScreen HIV-1 test, version 1.5 (AmpliScreen; Roche Molecular Systems Inc., Pleasanton, CA); Genetic Systems HIV-1 Western blot (Bio-Rad Laboratories, Redmond, WA); and Cambridge Biotech HIV-1 Western blot (Calypte Biomedical, Berkeley, CA). The Cambridge blot was used for the majority of specimens. The Cambridge and Genetic Systems blots have been shown to give concordant interpretations in overlap studies conducted to qualify for interchangeable use in our clinical laboratory. All commercial assays were performed using the standard assay protocol described in the package insert. However, there is one difference with regard to the EIA testing. We considered an EIA to be positive based on a single-well result, and samples were not rerun in duplicate. Repetition of an initially positive EIA is an intratest quality control safeguard against false-positive EIA results. The specificity of individual tests that we report (Table 1) may have been higher had we performed repeat testing. However, the difference has to be small, as there is very little room for improvement in the single-well EIA specificities.

Sources of specimens. Specimens from infected and uninfected U.S. blood/plasma donors ($n = 997$) and from international donors ($n = 64$) were obtained from BBI-SeraCare Diagnostics (West Bridgewater, MA). An additional 114 specimens were obtained from a CDC study conducted in Cameroon (25). Most of the international plasma samples (128 of 178) were from subjects infected with non-subtype-B HIV-1 as determined by genotyping, and the rest were negative or indeterminate for HIV infection. Domestic U.S. specimens were not necessarily genotyped and are presumed to be predominately of subtype B. A set of serial specimens ($n = 183$) from 15 seroconverting U.S. plasma donors was obtained from Zeptometrix, Inc. (Buffalo, NY). A panel of 34 HIV-2 specimens was obtained from BBI-SeraCare ($n = 2$) and from a CDC field site in Ivory Coast ($n = 32$). HIV-2 specimens had been verified by molecular techniques that distinguish HIV-2 from HIV-1. Specimens were unlinked from personal identifiers, and the CDC Institutional Review Board determined that their use represented "research not involving human subjects".

HIV-1 sensitivity/specificity panel. Specimens from the reference panel for evaluating HIV-1 sensitivity/specificity were assigned "true" infection status based on consensus negativity in screening tests or reactivity by Western blotting as follows. All specimens were tested by all serologic tests and NAAT. Specimens that were negative by all tests were defined as referent negative. If a specimen was reactive in any or all of these tests, a Western blot was performed. These specimens were defined as negative, indeterminate, or positive using the Association of State and Territorial Public Health Laboratory Directors-Centers for Disease Control (ASTPHLD-CDC) criteria for interpretation of the Western blot (4). Thus, all HIV-1 positives in the panel were confirmed positive by Western blotting ($n = 621$). All the negatives in the panel ($n = 513$) either were negative by all the screening tests ($n = 418$) or were Western blot negative ($n = 95$). Since the infection status of Western blot-indeterminate specimens ($n = 41$) cannot be defined serologically without follow-up, these specimens are not part of the sensitivity/specificity panel and are dealt with separately.

Seroconversion panels. Serial specimens were collected from donors early in the process of HIV-1 seroconversion. We tested 183 specimens from 15 donors. For the period from the first positive test (which was NAAT in all cases) to the development of a Western blot pattern that qualified as positive by ASTPHLD-CDC criteria (4), there were 126 specimens. For each test performed, we plotted the cumulative proportion of positive test results versus the time in days before the first positive Western blot test. We chose to plot results relative to the day of first Western blot-positive specimen rather than relative to the first positive NAAT specimen because there was less uncertainty in the time bracket over which a donor became Western blot positive. The interval between the first positive Western blot specimen and the preceding specimen was an average of 4.2 days (median, 2 days; range, 2 to 11 days). For the first positive NAAT, three donors did not have a previous negative specimen. Of the 12 remaining, the interval between first positive and the preceding negative NAAT was an average of 13.1 days (median, 5 days; range, 2 to 63 days).

TABLE 1. HIV tests evaluated in this study[a]

HIV test	Sensitivity	Specificity	Test category
GS HIV-1/2+O	99.8	99.4	Third-generation EIA
Abbott	99.4	97.7	Third-generation EIA
Vir HIV-1+O	99.7	99.0	Second-generation EIA
GS HIV-1/2 peptide	98.7	99.8	Second-generation EIA
GS rLAV	97.4	100.0	Second-generation EIA
Vir HIV-1	99.0	98.4	First-generation EIA
Oraquick	98.6	99.8	Rapid test
Reveal	99.0	99.8	Rapid test
Multispot	ND	ND	Rapid test
Uni-Gold	98.4	99.4	Rapid test
Procleix	97.4	99.6	NAAT
CDC RNA	95.8	99.4	NAAT
AmpliScreen	92.6	96.9	NAAT

[a] Sensitivity and specificity were evaluated for 621 HIV-1 reference-positive and 513 reference-negative specimens, respectively.

RESULTS

Sensitivity and specificity of individual tests. Test performance was evaluated on an HIV-1 sensitivity panel of 621 HIV-1-positive specimens and on a specificity panel of 513 HIV-1-negative specimens. All the antibody tests except one exceeded 98% sensitivity, and all but one exceeded 98% specificity (Table 1). Sensitivity of the three NAAT ranged from 93 to 97%. Specificity for two of the NAAT exceeded 99% (Procleix and CDC RNA) and was 96.9% for the third (AmpliScreen). For comparison of test performance between any two tests, differences of less than 1.3% in sensitivity and 1.4% in specificity were not statistically significant for this data set ($P > 0.05$; McNemar test with continuity correction).

Of the 621 positive sera, there were 128 sera from donors infected with non-B subtypes (25). These were initially intended to separately evaluate test performance on non-B subtypes. However, test performance was essentially the same in the non-B subset and the other specimens. The average difference in test sensitivity for the non-B and domestic specimens was 0.4%. Therefore, the sets are pooled for this analysis.

Dual testing algorithms. The performance of a dual testing algorithm on the sensitivity/specificity panel is shown for all 66 possible combinations of tests in the 12-by-12 table (Table 2). This algorithm requires that two tests register positive to re-

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TABLE 2. Specificity-optimized dual testing algorithm[a]

Test	Sensitivity and/or specificity of combination with[b]:											
	Third-generation EIA		Second-generation EIA			First-generation EIA Vir HIV-1	Rapid test			NAAT RNA		
	GS HIV-1/2+O	Abbott	Vir HIV-1+O	GS HIV-1/2 peptide	GS rLAV		Oraquick	Reveal	Uni-Gold	Procleix	CDC RNA	Ampli-screen
GS HIV-1/2+O	**99.8/99.4**	99.4	99.7	98.7	97.4	99.0	98.6	99.0	98.4	97.3	95.6	92.4
Abbott	99.6	**99.4/97.7**	99.2	98.6	97.1	98.9	98.4	98.9	98.2	97.3	95.7	92.4
Vir HIV-1+O	100.0	100.0	**99.7/99.0**	98.7	97.4	99.0	98.6	99.0	98.4	97.3	95.7	92.4
GS HIV-1/2 peptide	100.0	100.0	100.0	**98.7/99.8**	97.3	98.4	98.6	98.7	98.0	97.1	95.7	92.4
GS rLAV	100.0	100.0	100.0	100.0	**97.4/100**	97.3	97.1	97.3	96.8	95.7	94.2	91.0
Vir HIV-1	100.0	99.8	99.8	100.0	100.0	**99.0/98.4**	98.2	98.7	98.2	97.1	95.5	92.2
Oraquick	100.0	100.0	100.0	100.0	100.0	100.0	**98.6/99.8**	98.6	97.8	96.9	95.5	92.2
Reveal	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**99.0/99.8**	98.0	97.3	95.7	92.4
Uni-Gold	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**98.4/99.4**	96.4	94.8	91.2
Procleix	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**97.4/99.6**	95.7	92.6
CDC RNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**95.8/99.4**	91.43
AmpliScreen	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**92.6/96.9**

[a] The algorithm requires that specimens be concordantly reactive in two tests to be scored as positive (discordant and concordantly nonreactive results are scored as negative).

[b] Results in boldface along the diagonal indicate the sensitivity/specificity of the individual tests. Results above the diagonal are the sensitivities of the two-test combinations, and results below the diagonal are the specificities. Sensitivity and specificity were evaluated for 621 HIV-1 reference-positive and 513 reference-negative specimens, respectively.

port the specimen as positive. Discordant and dually negative test results are recorded as negative for HIV infection. In Table 2, the double entries along the diagonal are the sensitivities and specificities, respectively, of the individual tests. The sensitivities of the two-test algorithm are entered above the diagonal, and the specificities of the respective combinations are entered below the diagonal. Note that with this algorithm, the sensitivity of the two-test algorithm can be no higher than that of the individual tests and the specificity can be no lower. Also, the sensitivities and specificities are the same whether the testing procedure calls for testing all specimens with both tests (parallel testing) or whether the second test is performed only on specimens that are reactive in the first test (sequential testing).

The specificity of the two-test algorithm was 100% in all but 3 of 66 combinations, and none were significantly different. In all instances, the sensitivity of the combination of two third-generation EIA tests (99.4%) was higher than combinations of second- and first-generation tests (median, 98.4; range, 97.3 to 99.0) or combinations of rapid tests (median, 98.0; range, 97.8 to 98.6), but most of the comparisons were not significantly different. The sensitivities of NAAT combinations or combinations of NAAT with an antibody test were lower than those of the antibody test combinations, and most of the differences were statistically significant.

If the interpretive criteria of the two-test algorithm are altered so that discordant results are reported as positive instead of negative, there is a reciprocal increase in sensitivity and decrease in specificity (Table 3). The sensitivity increase is less than the specificity decrease. For the antibody test combinations, there is an average increase in sensitivity of 1.1% and decrease in specificity of 1.8%. We refer to the two dual test algorithms as the specificity-optimized (discordants equal a negative result) and the sensitivity-optimized (discordants equal a positive result) dual test algorithms (Tables 2 and 3).

Three-test algorithm. To close the "sensitivity gap" of the specificity-optimized two-test algorithm while preserving its specificity, we evaluated an algorithm in which discordant specimens are subjected to a third, "tie-breaking" test, the results of which determine the reporting status of the specimen. The first two tests are done in parallel (both must be done on all specimens), and concordant negatives and concordant positives are reported as such. The third test is done only if the first two tests are discordant. There are 660 possible three-test combinations. Selected examples are shown in Table 4. The sensitivities/specificities of three algorithms for each test combination are presented for comparison: the specificity-optimized two-test algorithm (as in Table 2), the sensitivity-optimized two-test algorithm (as in Table 3), and the three-test algorithm in which discordants are subjected to a third, tie-breaking test.

When the three EIAs with the highest sensitivity were part of the algorithms, the three-test algorithm resulted in preservation of the superior sensitivity of the sensitivity-optimized two-test algorithm with no change or a slight decrease in specificity compared to that seen for the specificity-optimized two-test algorithm (Table 4, lines 1 to 3). These differences were small improvements or compromises reflecting the fact that, for the three highest performing tests that were selected, there was little room for improvement over the performance of these tests individually or in dual combination. The differences are more compelling when the three EIAs with lower individual sensitivities are combined (Table 4, lines 4 to 6). Here, the three-test algorithm preserves the superior specificity and improves the sensitivity compared to what was seen for the specificity-optimized two-test algorithm. Also shown are examples of a three-test algorithm incorporating NAAT, similar to what is done in U.S. blood banks (Table 4, lines 7 to 9), and a three-test algorithm using rapid tests, similar to what would be done in an outreach, nonlaboratory setting (Table 4, lines 10 to

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TABLE 3. Sensitivity-optimized dual testing algorithm[a]

Test	Sensitivity and/or specificity of combination with[b]:											
	Third-generation EIA		Second-generation EIA			First-generation EIA Vir HIV-1	Rapid test			NAAT RNA		
	GS HIV-1/2+O	Abbott	Vir HIV-1+O	GS HIV-1/2 peptide	GS rLAV		Oraquick	Reveal	Uni-Gold	Procleix	CDC RNA	AmpliScreen
GS HIV-1/2+O	**99.8/99.4**	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100.0	100.0	100.0
Abbott	97.5	**99.4/97.7**	99.8	99.5	99.7	99.5	99.5	99.5	99.5	99.5	99.5	99.5
Vir HIV-1+O	98.4	96.7	**99.7/99.0**	99.7	99.7	99.7	99.7	99.7	99.8	99.8	99.8	99.8
GS HIV-1/2 peptide	99.2	97.5	98.8	**98.7/99.8**	98.9	99.4	98.7	99.0	99.1	99.0	98.9	98.9
GS rLAV	99.4	97.7	99.0	99.8	**97.4/100**	99.2	98.9	99.2	98.9	99.2	99.0	99.0
Vir HIV-1	97.9	96.3	97.7	98.2	98.4	**99.0/98.4**	99.4	99.4	99.3	99.4	99.4	99.4
Oraquick	99.2	97.5	98.8	99.6	99.8	98.2	**98.6/99.8**	99.0	99.1	99.0	98.9	98.9
Reveal	99.2	97.5	98.8	99.6	99.8	98.2	99.6	**99.0/99.8**	99.5	99.2	99.2	99.2
Uni-Gold	99.0	97.3	98.8	99.2	99.4	97.8	99.2	99.2	**98.4/99.4**	99.3	99.1	99.1
Procleix	99.0	97.3	98.6	99.4	99.6	98.1	99.4	99.4	99.0	**97.4/99.6**	97.6	97.4
CDC RNA	98.8	97.1	98.4	99.2	99.4	97.9	99.2	99.2	98.8	99.0	**95.8/99.4**	96.9
AmpliScreen	96.3	94.5	95.9	96.7	96.9	95.3	96.7	96.7	96.3	96.5	96.3	**92.6/96.9**

[a] Algorithm requires that specimens be reactive in either one or both of the two tests in order to be scored as positive (concordantly nonreactive results are scored as negative).

[b] Results in boldface along the diagonal indicate the sensitivity/specificity of the individual tests. Results above the diagonal are the sensitivities of the two-test combinations, and results below the diagonal are the specificities. Sensitivity and specificity were evaluated for 621 HIV-1 reference-positive and 513 reference-negative specimens, respectively.

12). In all these cases, the three-test algorithm improves sensitivity to a greater extent than it decreases specificity relative to the two-test algorithms. For the data in Table 4, sensitivities and specificities of the three-test algorithm were higher by averages of 0.9% and 1.3%, respectively, than the less sensitive or less specific two-test algorithm. On the other hand, the sensitivities and specificities of the three-test algorithm were decreased by only 0.3% and 0.1% compared to the more sensitive and specific dual test algorithms. Note also that the sensitivity and specificity of the three-test algorithm is the same regardless of the order in which the three tests are performed. Since the third test is done on fewer than 1% of specimens, one could select the most expensive test for this spot.

Sensitivity of tests in early infection. The sensitivities of selected tests for early infection relative to the time of Western blot-defined HIV-1 infection are shown in Fig. 1. The sequence of reactivity was compared by ranking the tests in the order in which the cumulative frequency of positive results for each test was 50%. The NAAT, Procleix, was positive approximately 26 days before the Western blot registered positive and 12 days before the earliest EIA registered positive. The third-generation EIAs, GS HIV-1/2+O and Abbott, were reactive 14 and 12 days, respectively, before the blot was positive. The blot became indeterminate about 9 days before it became positive. The second-generation EIA, GS rLAV, was positive 6 days before the blot was positive, and the first-generation EIA, Vir HIV, was positive 1 day after the blot was positive. Two rapid tests, Reveal and Multispot, were positive about the same time the blot became indeterminate and 9 and 7 days before the blot became positive. Two other rapid tests, Oraquick and Uni-Gold, were positive about the same time the blot became positive. A similar sequence of reactivity was found when the

TABLE 4. Three-test (tie-breaking) algorithm[a]

Test 1	Test 2	Test 3 (tie breaker)	Two-test algorithm (specificity optimized):		Two-test algorithm (sensitivity optimized):		Three-test algorithm (tie breaker):	
			Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
GS HIV-1/2+O	Abbott	Vir HIV-1+O	99.4	99.6	99.8	97.5	99.8	99.6
GS HIV-1/2+O	Vir HIV-1+O	Abbott	99.7	100.0	99.8	98.4	99.8	99.6
Abbott	Vir HIV-1+O	GS HIV-1/2+O	99.2	100.0	99.8	96.7	99.8	99.6
GS HIV-1/2 peptide	GS rLAV	Vir HIV-1	97.3	100.0	98.9	99.8	98.7	100.0
GS HIV-1/2 peptide	Vir HIV-1	GS rLAV	98.4	100.0	99.4	98.2	98.7	100.0
GS rLAV	Vir HIV-1	GS HIV-1/2 peptide	97.3	100.0	99.2	98.4	98.7	100.0
GS HIV-1/2+O	Abbott	Procleix	99.4	99.6	99.8	97.5	99.4	99.6
GS HIV-1/2+O	Procleix	Abbott	97.3	100.0	100.0	99.0	99.4	99.6
Abbott	Procleix	GS HIV-1/2+O	97.3	100.0	99.5	97.3	99.4	99.6
Oraquick	Reveal	Uni-Gold	98.6	100.0	99.0	99.6	98.7	100.0
Oraquick	Uni-Gold	Reveal	97.8	100.0	99.1	99.2	98.7	100.0
Reveal	Uni-Gold	Oraquick	98.0	100.0	99.5	99.2	98.7	100.0

[a] In the three-test algorithm, specimens are initially tested with two tests. Concordantly reactive specimens are scored as positive, concordantly nonreactive specimens are scored as negative, and discordant specimens are tested with a third test, the results of which determine the assignment of positivity or negativity. Sensitivity/specificity results of the dual testing algorithms (from Tables 2 and 3) are shown for comparison. Sensitivity and specificity were evaluated for 621 HIV-1 reference-positive and 513 reference-negative specimens, respectively.

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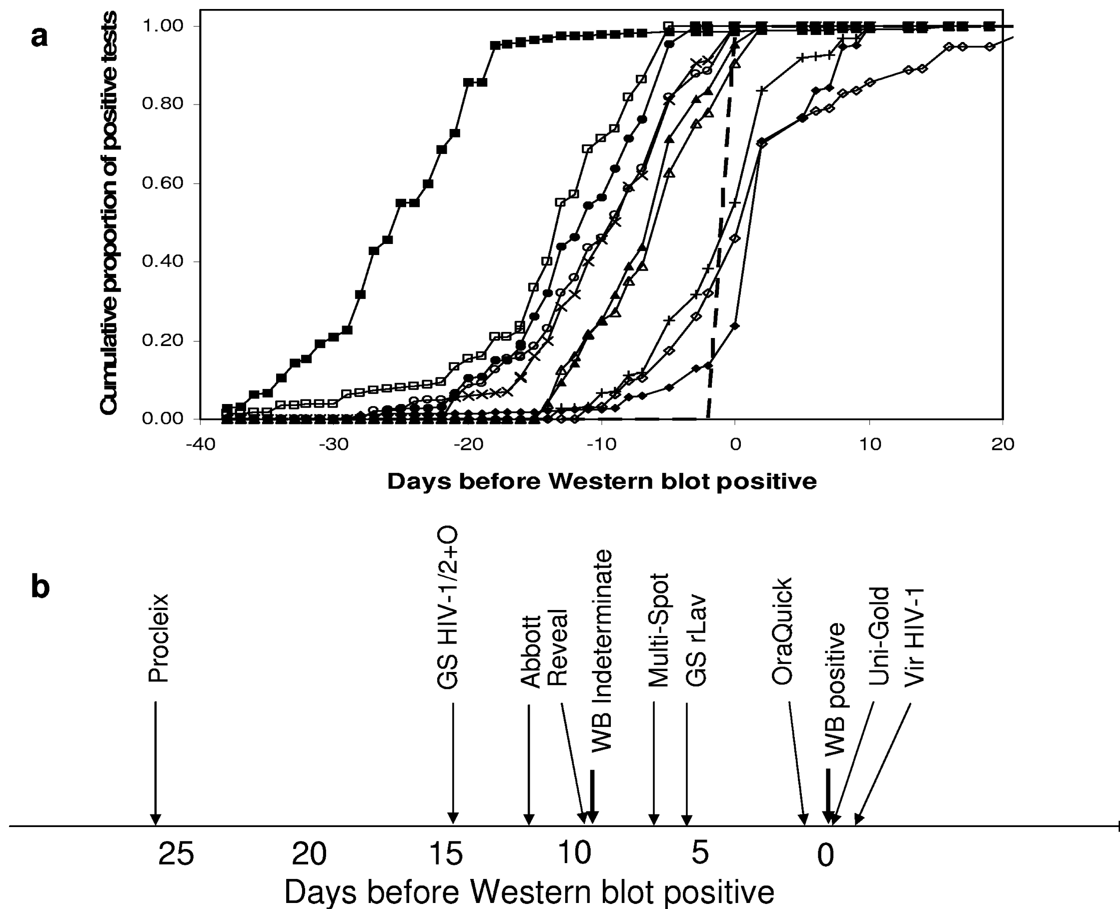

FIG. 1. Evolution of test reactivity during early HIV-1 infection. (a) Cumulative frequency of positive test results relative to the number of days before the Western blot was first positive. Serial specimens (*n* = 183) were collected during seroconversion from 15 donors and tested by the following tests: Procleix (■), GS HIV-1/2 (□), Abbott (●), Reveal (○), indeterminate Western blotting (×), Multispot (▲), GS rLAV (△), Oraquick (+), positive Western blotting (—-), Uni-Gold (◇), and Vir HIV-1 (◆). (b) Sequence along timeline in which the indicated tests registered 50% of specimens positive. WB, Western blot.

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data were plotted relative to the time in days since the first NAAT-positive specimen (data not shown).

HIV-2 and group O detection. A panel of 34 HIV-2 specimens was tested. All the tests that are licensed for the detection of HIV-2 antibody (GS HIV-1/2+O, Abbott, GS HIV-1/2 peptide, OraQuick Advance, and Multispot) were reactive with all of the 34 HIV-2 specimens. For the other tests, the detection rate was variable: for Vir HIV-1+O, 34 of 34; for Uni-Gold, 34 of 34; for Reveal, 34 of 34; for Vir HIV-1, 29 of 34; and for GS rLAV, 16 of 34. As expected, none of the NAAT detected HIV-2.

Four specimens from HIV group O-infected patients (confirmed by sequence) were available for testing. Three were Western blot positive and one was indeterminate. Most of the serologic assays (GS HIV-1/2+O, Abbott, Vir HIV-1+O, GS HIV-1/2 peptide, Vir HIV-1, and Reveal) were reactive with four of the four plasma samples. GS rLAV and Oraquick were reactive with three of four, and Uni-Gold was reactive with one of three (the fourth serum sample was not tested by Uni-Gold). The Procleix (Aptima) NAAT was reactive with all four plasma samples.

Indeterminate Western blots. There were 41 indeterminate blots encountered in the assembly of the sensitivity/specificity panels. Four of the indeterminate specimens were positive in eight or nine of the nine serologic assays and in all three NAAT. They would have registered positive or at least been flagged as discordant by most of the alternative algorithms. The remaining 37 indeterminates were positive in only one test (*n* = 23), in two tests (*n* = 9), in three tests (*n* = 3), or in four or five tests (*n* = 1 each). All these specimens were negative by the NAAT. Most of these would have registered as concordantly negative in most of the algorithms.

DISCUSSION

Two panels were established for HIV-1 detection, a sensitivity/specificity panel consisting of HIV-1-positive and -negative plasma samples and a seroconversion panel consisting of serial blood samples from patients with acute HIV-1 infection. Negative plasma samples either were negative by all HIV assays or, if positive by any test, were negative by Western blotting. Designated positive plasma samples were all positive by

Western blotting. In the seroconversion panel, all patients ultimately became Western blot positive. Thus, the panels are defined by reference to a unique composite standard that relies in large part on Western blot results for definitive designation of specimen status. The Western blot has traditionally been the gold standard against which HIV assays are compared. It should be noted, however, that false-positive Western blots have been reported (7, 17, 32, 40) and that when comparing tests to a gold standard, the best a test can do is match the Western blot results. If the test is actually better or if the Western blot is incorrect in some instances, a more accurate test would look worse.

Another caution relates to the statistical analyses. Most differences in test or algorithm performance were small and incremental, as expected for tests with excellent individual performance. In general, differences in sensitivity or specificity of greater than 1.3% or 1.4% were statistically significant differences, but these data were not corrected for multiple comparisons. This study was not intended to be a comparison of test performance in a particular algorithm. Rather, we compared algorithm strategies using current test combinations to assess the relative advantages and magnitude of the differences between algorithm strategies.

Three multitest algorithm strategies were evaluated. These can be viewed as specificity-optimized, sensitivity-optimized, and tie-breaking algorithms. The two dual-test algorithms differ in the interpretation of discordant test results. If both tests are required to be concordantly positive to be scored as positive (discordance equals a negative result), specificity is optimized (Table 2). This strategy is in principle similar to the current U.S. PHS-recommended algorithm with the proviso that discordant specimens may be reported as indeterminate or negative (4, 6, 24, 27). Conversely, if discordants are scored as positive, sensitivity is optimized (Table 3). Overall, the average differences in sensitivity and specificity between the two dual-test algorithms for all combinations tested were 2.7% and 1.8%, respectively (1.1% and 1.4% for the serologic tests only). The specificity-optimized algorithm could be performed sequentially (the second test is done only if the first test is positive). In the sensitivity-optimized algorithm, both tests would have to be run on all specimens. The three-test algorithm represents a favorable compromise between the two dual-test algorithms with an improvement in sensitivity greater than the loss of specificity relative to the specificity-optimized dual-test algorithm and, conversely, an improvement in specificity greater than the loss of sensitivity relative to the sensitivity-optimized dual-test algorithm (Table 4).

The evaluation of performance in Western blot-defined HIV-1-positive and -negative panels omits the evaluation of Western blot-indeterminate specimens, because without follow-up, the infection status of these specimens is unknown (or undefined). The causes of indeterminate patterns on Western blot include technical artifact, laboratory error, irrelevant cross-reactions, nonspecific binding, infection with a related retrovirus such as HIV-2, the presence of an antigenic variant of HIV-1, early HIV-1 infection, or late-stage disease (3, 6, 7, 17, 32, 40). Antiretroviral therapy reduces viral load and has been reported to reduce HIV-specific antibody (11, 16, 22, 23, 28). Presumably, patients on antiretroviral therapy would be diagnosed before the initiation of therapy. There was no indication that the current tests were less sensitive for infection with non-B subtypes or variants. The likelihood that an indeterminate blot reflects true infection rises with higher HIV prevalence in the test population. In low-prevalence settings, most indeterminate blots are not from infected people (3, 13, 17, 26, 39). Of the 41 indeterminate blots encountered in this study, four were positive in the majority of the serologic assays and in all three NAAT. They would have registered positive or at least been flagged as discordant by most of the alternative algorithms. The remaining 37 indeterminates were negative in most of the serologic tests and in all three NAAT. They would have registered as concordantly negative by most test combinations and thus been subsumed into definitive negative results by most of the algorithms. This is consistent with the expectation that most Western blot-indeterminate specimens do not represent bona fide infection. However, without confirmed designation of infection status by follow-up, it is conjecture to evaluate the performance of tests on these specimens. As an alternative, we assembled panels of sera that frequently register an indeterminate blot result and are from infected people, i.e., specimens collected serially from patients with newly acquired HIV-1 infection and specimens from patients with HIV-2 infection. We do not have the converse type panel: indeterminate blots from people known by follow-up not to be infected. This type of panel is difficult to assemble. It requires follow-up and would be biased by the test or tests used for initial screening.

In the evaluation of the seroconversion panel, the sequence and intervals of seroconversion are consistent with other studies (5, 8, 20). Since the sample size was small (183 specimens from 15 donors), the intervals between test reactivities may not be precise. However, since all tests were run on all specimens, the ranking or sequence of reactivity is comparable between tests. The Western blot was indeterminate about 9 days before it was positive (Fig. 1). Most of the tests were already positive or became positive during this time, but there were differences in the analytic sensitivity as measured by comparison of how early the tests became reactive. The NAAT, which in the panel of established infection was less sensitive than the serologic tests, was the most sensitive for early infection, reflecting the fact that viral replication precedes seroconversion. The third-generation EIAs were decidedly more sensitive than the earlier-generation EIAs and than two of the four rapid tests. For tests that are positive before the Western blot becomes indeterminate or positive, algorithms employing these tests would have a substantial advantage over the conventional Western blot-based algorithm in diagnosing early infection or flagging it for further testing. The overall effect on HIV detection in a given diagnostic setting would depend on how many specimens in the test population were from early infection. Higher-prevalence, emerging-epidemic, and higher-risk settings are likely to have more. Some indication of the relative numbers of early infection and established infection can be gleaned from surveys for primary HIV infection. In these studies, the population is screened by EIA, and EIA-negative specimens are screened by NAAT (2, 26, 33, 35, 36, 36, 37, 39). The incremental yield of HIV-1 infections detected over that of serology alone ranges from 0 to 11% (33, 35–37, 39).

The potential role of NAAT in a diagnostic algorithm reflects the unique features of this technology. Ideally, screening

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and supplementary tests should be orthogonal; that is, they should differ sufficiently in format or content such that they are not prone to the same false-positive or false-negative effects. NAAT is an appealing addition in that it detects virus directly and uses technology that does not share features with the antibody tests. However, it is less sensitive for detection of established infection than the serologic tests (Table 1) (1, 19, 30, 42). On the other hand, it is more sensitive than the serologic tests for early infection (Fig. 1) (1, 2, 8, 12, 20, 32). Our data support the use of NAAT as a supplementary test for confirming antibody-positive sera and as a screen of antibody-negative sera for primary infection. To date, NAAT diagnostics has been reserved for niche applications where antibody is not present (detection of primary HIV infection) or the presence of HIV antibody is uninformative (HIV diagnosis in infants). U.S. blood banks use NAAT to screen EIA-negative specimens for primary HIV infection. They also may use NAAT testing in their standard algorithm for evaluation of EIA-reactive screening tests (2, 26, 39). If the NAAT is positive on the EIA-reactive specimen, infection is confirmed without the need for a Western blot. If NAAT is negative, the specimen is considered unresolved and undergoes further testing by the conventional algorithm (i.e., Western blotting). This is analogous to the three-test algorithm, where discordant results on the first two tests are resolved by a third test (Table 4, line 8 or 9). The data in Table 4 indicate that an EIA tie-breaker would function as well as the Western blot and not as well if discordants (EIA positive, NAAT negative) were registered as negative (Table 4, compare lines 8 and 9, specificity-optimized dual-test algorithm with the three-test algorithm).

Regarding HIV-2 detection, all the tests that have an HIV-2 designation registered 34 of 34 HIV-2 specimens positive. For the other tests, the detection rate was variable (16 to 34 of 34). The NAAT do not detect HIV-2. In the current U.S. PHS algorithm, specific testing for HIV-2 is prompted by an indeterminate HIV-1 Western blot result for an EIA-reactive specimen or by clinical suspicion (specimens from symptomatic or exposed patients with links to West Africa) (9, 10, 21, 27, 34). This is not an entirely satisfactory process. Specimens from dually infected people would be reported as HIV-1 positive, and, rarely, HIV-2 specimens that are HIV-1 Western blot negative rather than indeterminate do occur. Combination HIV-1/2 tests target both HIV-1 and HIV-2, have high sensitivity for HIV-2, and do not rely on cross-reactivity with HIV-1. Thus, HIV-2 specimens would be expected to cotrack with HIV-1 specimens in most of the algorithms presented here without a pattern that could be used as a flag for HIV-2 testing. If HIV-2 is a concern, all specimens that register positive in an alternative algorithm would have to be tested for HIV-2 with a discriminatory test such as Multispot. This may actually require less overall HIV-2 testing than is done in the conventional algorithm, where indeterminate Western blots are tested. This is because in low-prevalence settings, indeterminate blots generally greatly outnumber positive blots (3, 17, 26, 39).

Given the anticipated prevalence of established HIV-1 infection, early HIV-1 infection, and HIV-2 infection and the sensitivities/specificities, the data presented here may be used to project the algorithm accuracy, the number of tests required, and the cost of the respective algorithms for a given diagnostic setting. There is no recommended standard for acceptable algorithm performance. The FDA draft guidance for manufacturers seeking licensure of individual tests recommends demonstration that the lower bound of the one-sided 95% confidence interval for sensitivity and specificity exceed 98%. For the sample size in our panel, this would require that the measured sensitivity and specificity exceed 98.6% and 98.8%, respectively. The test combinations exceeded this in at least one of the algorithm strategies, and most exceeded it in all strategies. As an alternative to minimum acceptable criteria, comparative algorithm performance could be used for the selection of appropriate diagnostic procedures. In general, for any given test combination, the three-test algorithm results in the highest net combination of sensitivity/specificity. Conversely, for any given algorithm, test combinations that include third-generation EIAs result in the highest sensitivity/specificity (Tables 2 to 4). However, a number of other test combinations or algorithms have performance that is not significantly inferior. Thus, from the standpoint of minimum performance criteria or of relative performance, these data support the implementation of alternate algorithms that do not include the Western blot, that result in less-ambiguous testing (discordants or indeterminates), that cost less, and that can accommodate special features or a testing program such as on-site (outreach) testing and screening for acute HIV infection.

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