

HIV TESTING ALGORITHMS

A STATUS REPORT

A PUBLICATION FROM THE ASSOCIATION OF PUBLIC HEALTH LABORATORIES
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Status of the Status Report

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HIV Testing Algorithms: A Status Report outlines a menu of testing algorithms, for both point-of-contact and laboratory settings based on the testing technology and data available in 2009. Since its publication, there have been several developments in the field of HIV diagnostics including the FDA-approval of antigen/antibody combination assays and the evaluation of new data.

At the 2010 HIV Diagnostics Conference, data were presented that addressed many of the algorithm data needs outlined in the Status Report. At the conclusion of the conference, a new laboratory algorithm¹ was proposed for serum/plasma testing that addresses some of the shortcomings of the Western blot. This strategy begins with antigen/antibody combination assays or sensitive antibody assays for screening, followed by HIV-1/2 discriminatory antibody testing and nucleic acid amplification testing (NAAT) for specimens with a reactive screening test and negative antibody discriminatory assay. The performance of the strategy is currently being evaluated. This algorithm has the potential to identify infections earlier, differentiating HIV-1 from HIV-2 and decreasing turnaround time. Because this strategy requires serum/plasma specimens, the algorithm outlined in the Status Report for oral fluid and dried blood spot specimens (Laboratory Algorithm 1) is unchanged. The point-of-contact algorithms proposed in the Status Report require further evaluation.²

The Status Report was a great step forward in the process of updating the algorithm, and it remains an important source of historical information regarding the rationale for diagnostic strategies. The approval of antigen/antibody combination (fourth generation) immunoassay technology and the proceedings of the 2010 HIV Diagnostics Conference have led to significant progress in the development of HIV testing strategies that update those in the Status Report.

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1. Presentation is available at www.hivtestingconference.org/hivtesting2010/PDF/Presentations/BransonSummary.pdf
 2. The conference proceedings and a detailed conference summary can be viewed at www.hivtestingconference.org.

Update

March 2010

Since *HIV Testing Algorithms: A Status Report* was published in April, 2009, several developments have occurred that necessitated an update to the report. At several points in the laboratory algorithms section of the report (pp. 25-31, 32, 39, 41), the April 2009 version stated that a plasma specimen is required to conduct HIV nucleic acid amplification testing (NAAT). The qualitative RNA NAAT that is FDA-approved as a supplemental test can now be used with serum specimens as well as plasma. Several instances in the Status Report (pp. 25, 29, 38, 39) also state that it may be necessary to repeat a positive NAAT for confirmation. This is only recommended with tests that are not FDA-approved for supplemental testing, or with specimens that are negative for HIV antibody.

To avoid any confusion between the original and updated versions of the status report, additions and changes to the text have been highlighted; deleted text has been struck through.

HIV Testing Algorithms: A Status Report

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Executive Summary

HIV Testing Algorithms: A Status Report

In 1989 the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released recommendations for a sequential, two-test algorithm for serodiagnosis of HIV-1 infection. In this algorithm, screening is conducted with an enzyme immunoassay (EIA). Specimens repeatedly reactive by EIA are subjected to a more specific supplemental test, either a Western blot (WB) or an indirect immunofluorescence assay (IFA). Over the past two decades, many advances in HIV diagnostics have been achieved: less complex rapid tests have been FDA-approved for screening at the point of contact (POC) with the patient, more sensitive and specific laboratory-based tests are available, and more complex rapid tests can detect HIV-2.

The “HIV Testing Algorithms: Status Report” describes a menu of HIV testing algorithms that have the potential to augment and provide alternatives to the algorithm currently used to diagnose HIV infection. APHL and CDC convened two working groups of HIV diagnostic subject matter experts to develop these new algorithms. This report describes algorithms to use in POC and laboratory settings, as well as supporting evidence, limitations, and additional data needed to substantiate the algorithms as of April 2009. This document does not contain recommendations from either CDC or APHL.

PROPOSED TESTING STRATEGIES FOR POC RAPID HIV TESTING

Most POC testing will be conducted with rapid HIV tests that are “waived” under the Clinical Laboratory Improvement Amendments of 1998 (CLIA).

- **Algorithm 1: Single Rapid Test (A1) for HIV Screening.**
This is the current algorithm, in which a single test is performed with an oral fluid or blood specimen. If the rapid test (A1) is reactive, a “preliminary positive” result for the presence of HIV-1 and/or HIV-2 antibodies is reported. A specimen should be collected for supplemental laboratory testing to confirm the results. If the initial test (A1) is non-reactive, a “negative” result for HIV-1 and/or HIV-2 antibodies is reported.
- **Algorithm 2: Two Rapid Tests (A1/A2) Performed in Sequence on Blood.**
Two different rapid test products are used sequentially, both on blood specimens, to improve the positive predictive value of POC testing. If rapid test A1 is non-reactive for HIV-1 and/or HIV-2 antibodies, a “negative” result for HIV-1 and/or HIV-2 antibodies is reported. If test A1 is reactive, a second test (A2) from a different manufacturer is performed. If both A1 and A2 are reactive, the result is reported as: “Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If A1 is reactive and A2 is non-reactive, the result is reported as: “Inconclusive rapid test result; requires additional testing.”
- **Algorithm 3: Two Rapid HIV Tests (A1 oral/A2 blood) Performed in Sequence; If A2 Is Negative, A1 Is Repeated on Blood.**

In this algorithm, a reactive test performed on oral fluid (A1) is followed by a test from a different manufacturer (A2) performed on blood. If both A1 and A2 are reactive, the result is reported as: “Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If the A2 test is nonreactive, the A1 test is repeated, but this time on a blood specimen. If the A1 test is reactive with both oral fluid and blood but the A2 test is non-reactive with blood, these results may represent false-positive A1 test results with two different specimen types or a false-negative A2 result. This should be reported as: “Inconclusive rapid test result; requires additional testing.” If the A1 test on the oral fluid specimen is reactive, but both blood tests (A2; follow-up A1) are non-reactive, then the result should be reported as: “Negative for HIV-1 and HIV-2 antibodies.” In this case the oral fluid test is considered a false-positive result and the blood tests are considered true-negative results.

- **Algorithm 4: Three Rapid HIV Tests (A1/A2/A3) Performed in Sequence on Blood. (A1, A2 and A3 must be different rapid tests.)**

The strategy of using three different blood tests in sequence has been proposed as a way to confirm results at the POC, but requires further validation before implementation. If A1 is reactive, another blood specimen should be obtained to perform test A2. If A2 is also reactive, the result is reported as: “Presumptive positive for HIV-1 and/or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If the A2 test is negative, then another blood specimen should be tested using a different product, A3. If A3 is reactive, in conjunction with a reactive A1 result and a non-reactive A2 result, then the test results should be reported as: “Presumptive positive for HIV-1 and/or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If A3 is negative, in conjunction with a reactive A1 result and a negative A2 result, the result should be reported as: “Inconclusive rapid test result; requires additional testing.”

PROPOSED TESTING STRATEGIES FOR LABORATORY HIV TESTING

In general, laboratories use assays categorized by CLIA as high complexity. However, some laboratories may choose to use rapid HIV tests (categorized under CLIA as moderately complex when used with serum or plasma) as one of the component assays of the laboratory testing algorithm.

- **Algorithm 1: HIV-1 Only Immunoassay, With Supplemental NAAT Option.**

This algorithm most closely reflects the current algorithm, a stand-alone HIV-1 immunoassay as the (A1) screening test followed by a (B1) supplemental test, either a Western blot (WB) or an indirect immunofluorescence assay (IFA). Samples that are repeatedly reactive using A1 are then tested with the B1 supplemental test; if B1 is also positive, the test results are reported as positive for HIV. Alternatively, after a repeatedly reactive A1 test, an individual nucleic acid amplification test (NAAT) (B2) can be used as a supplemental test for confirmation. NAAT (B2) can also be used to resolve negative or indeterminate WB or IFA (B1) results. If NAAT (B2) is negative but the sample was repeatedly reactive on the A1 test, B1 testing by WB or IFA is still required. If an acute HIV infection is suspected, refer to Algorithm 4 for guidance.

- **Algorithm 2: HIV-1/HIV-2 Immunoassay, With Supplemental NAAT Option.**

The inclusion of antigens designed to detect HIV-2 in FDA-approved diagnostic tests is increasingly common. Algorithm 2 offers the option of using B1 (HIV-1 WB or IFA) or B2 (HIV-1 NAAT) as a supplemental test on a specimen that is repeatedly reactive using A1. If B2 (NAAT) is not performed initially, it may be used to resolve indeterminate or negative B1 (WB or IFA) results. As in Algorithm 1, if B2 (NAAT) is negative but follows a repeatedly reactive A1, B1 testing (either a WB or IFA) is required. Algorithm 2 has the potential to detect more early-stage infections, but a negative NAAT result does not rule out infection: specimens that are reactive on an HIV-1/2 combo assay, but negative or indeterminate on supplemental testing, could represent HIV-2 infections. (See Algorithm 5 for more information). If an acute HIV infection is suspected, refer to Algorithm 4 for more information.

- **Algorithm 3: Dual HIV-1/HIV-2 Immunoassay.**

Dual immunoassays can potentially maximize the sensitivity of the algorithm to detect early and long-standing HIV infections while maintaining or improving specificity compared with the traditional EIA/WB algorithm. If both assays have similar sensitivity and specificity, the dual immunoassay could reduce the number of discordant results that occur with Algorithms 1 and 2. Algorithm sensitivity is dictated by the initial screening assay, thus the EIA or chemiluminescent immunoassay (CIA) with the best sensitivity should be used as A1. A negative A1 result is proposed to be reported as negative for HIV-1 and HIV-2 antibodies. (If acute HIV infection is suspected, Algorithm 3 should be followed with Algorithm 4.) The A2 test must be a different EIA or CIA than A1, with different antigen properties or binding/detection methods, to minimize concurrent non-specific reactivity in uninfected patients. If A1 and A2 are repeatedly reactive, the result is reported as: “Presumptive Positive for HIV-1 and/or HIV-2 antibodies and requires medical follow-up for further evaluation and testing.”

- **Algorithm 4: Acute HIV Infection Testing.**

Detecting HIV infection as early as possible after transmission can provide valuable information to patients and potentially prevent the spread of HIV. Testing of individual specimens by NAAT is useful for patients who have symptoms of acute retroviral infection and report recent high-risk exposure. The purpose of Algorithm 4 is to detect HIV-1 RNA in specimens with negative antibody results from an EIA, CIA or rapid test by either pooled or individual NAAT.

- **Algorithm 5: HIV-2 Testing.**

HIV-2 testing should be considered in cases where a client has potential epidemiologic risk factors for HIV-2 (i.e., sex partners from countries where HIV-2 is endemic, sex partners known to be infected with HIV-2, blood transfusion or non-sterile injection in countries where HIV-2 is endemic, needle sharing with a person from an HIV-2 endemic country or with a person known to be infected with HIV-2, children of women who have risk factors for HIV-2), clinical suspicion of AIDS in the absence of a positive test for antibodies to HIV-1; or an HIV-1 Western blot with unusual indeterminate patterns, such as Gag p55, p24 or p17 plus Pol bands p66, p51 (RT) or p31/32 (integrase). At the time this status report was written, there was no FDA-approved supplemental test for HIV-2 confirmation.

There are many challenges associated with implementing the proposed algorithms, ranging from structural (policy, law) to operational (staff training, developing quality assurance protocols). The ultimate goal is to have an increased number of individuals tested accurately and, if infected, linked into medical care as soon as possible.

Key to Acronyms and Symbols

+ = Reactive; or, positive

- = Non-Reactive; or, negative

CI = confidence intervals

CIA= Chemiluminescent Immunoassay

EIA= Enzyme immunoassay

IFA=Indirect immunofluorescence assay

HIV-1/HIV-2 = HIV-1/2

NAAT= Nucleic Acid Amplification (HIV-1 RNA) Test

NPV = negative predictive value

POC = point of contact

PPV = positive predictive value

RR = repeatedly reactive

S/CO = signal-to-cutoff ratio

WB= Western blot

HIV Testing Algorithms: A Status Report

Purpose

In 1989 the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released recommendations for a sequential, two-test algorithm to produce a serodiagnosis of HIV-1 infection.¹ In this algorithm, the initial screening is conducted with an enzyme immunoassay (EIA). Specimens that are repeatedly reactive by EIA are subjected to a more specific supplemental test, either a Western blot (WB) or an indirect immunofluorescence assay (IFA).

Since then, the sensitivity of HIV screening tests has increased. Many tests are capable of detecting HIV-2 infections.² Less complex rapid tests are approved for use at the point of contact (POC) with the patient, with oral fluid, finger-stick or venipuncture whole blood specimens.³ These and other more complex rapid tests can detect HIV-2 and may even distinguish HIV-1 from HIV-2 infections.¹ In recognition of these advances, APHL and CDC convened two working groups of multi-disciplinary, HIV diagnostic subject matter experts to develop alternatives to the testing algorithm recommended in 1989.

This report describes the proposed HIV-testing algorithms, for use in POC and laboratory settings, and provides supporting evidence, limitations and the additional data needed to substantiate each algorithm as of April 2009. This document does not contain recommendations from either CDC or APHL. The “HIV Testing Algorithms: Status Report” is targeted to public health, clinical and commercial laboratorians; HIV/AIDS prevention program managers; and others involved in HIV testing. The authors’ intent is to encourage HIV testing programs to validate one or more of the alternative algorithms to obtain additional performance data; to identify feasible and appropriate situations for specific testing strategies and algorithms; and to recognize and describe operational and policy challenges associated with adoption of alternative algorithms in order to plan for appropriate training and technical assistance.

Additional studies and data gathering are necessary to substantiate the algorithms proposed in this document. If laboratories and POC facilities implement one of the report’s proposed algorithms, testing personnel must also follow the current algorithms in parallel for validation. Caution should be used during validation, as there is no perfect gold standard for HIV testing.⁴ Laboratorians and program managers should work together closely during the implementation of a new algorithm to ensure consistent, reliable and integrated HIV testing between POC and laboratory.

Background

Following their introduction in the mid-1980s, data from newly introduced HIV diagnostic tests accumulated. CDC and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), now known as APHL, held hosted annual human retrovirus consensus conferences throughout the late 1980s and early 1990s to analyze test performance and assess

potential testing strategies. The 1989 CDC and APHL recommendations and subsequent guidelines for serologic testing with combination HIV-1/HIV-2 (HIV-1/2) screening EIA, published in 1992², resulted from these consensus conferences.

Since this time, evaluations of new HIV screening tests have led to their implementation in a variety of settings. WB and IFA supplement tests used for confirmation are less sensitive in early HIV infection than some of the newer screening tests, increasing the potential for negative, indeterminate or inconclusive results with some specimens from HIV-infected persons.^{5,6} In addition, nucleic acid amplification tests (NAAT) have been approved in the United States for direct detection of HIV RNA, which may be present before antibodies develop.⁴

The US Food and Drug Administration (FDA) continues to evaluate new HIV tests. All of the current, FDA-approved HIV diagnostics are listed on the agency's Center for Biologics Evaluation and Research website,⁷ which is updated regularly.

The types and number of available HIV tests have expanded substantially since the current testing algorithm was approved in 1989. To reflect this progress, updated strategies for diagnostic testing are needed. In January 2004, APHL and CDC created an HIV Steering Committee to monitor key developments in diagnostic testing, facilitate the timely exchange of information, and develop new recommendations as needed. Developing new HIV testing algorithms has been a priority of APHL and CDC, and a top priority of the HIV Steering Committee.

Since August 2006, two multi-disciplinary workgroups under the direction of the steering committee have worked to develop proposals for the best combination of assays to determine HIV infection status in laboratory and point-of-contact (POC) settings. The laboratory algorithm workgroup includes representatives from commercial, clinical and public health laboratories, APHL, American Society for Microbiology (ASM), American Clinical Laboratories Association (ACLA), blood banks, CDC, College of American Pathologists (CAP), Department of Defense (DOD) and the FDA. The POC algorithm workgroup is comprised of representatives from APHL, CDC, FDA, the National Alliance of State and Territorial AIDS Directors (NASTAD), state and local public health laboratories, and state and local public health HIV/AIDS prevention programs.

Typically, the desired outcome of POC HIV testing is to efficiently distinguish the uninfected persons from those most likely to be infected and in need of further medical evaluation, care and treatment. Where POC testing is provided, accuracy is a key focus. A higher sensitivity will minimize the number of false negatives; thus decreasing the number of missed diagnoses. The use of two or more tests can identify possible false positive results and improve the quality and accuracy of the testing.

The objective of testing in the laboratory is similar, with the goal to provide as much accurate information as possible to help the clinician make a definitive diagnosis. In laboratory-based testing, sensitivity and specificity are equally important.

To date, most HIV diagnostic tests have relied upon the detection of antibodies. The interval between infection with HIV and the development of detectable antibodies is known as the window period. The importance of this phase, defined clinically as acute infection, has become

increasingly apparent. During acute infection, persons are usually unaware they are infected, but high levels of viremia in this phase increase the per-act risk of transmitting HIV infection. Thus, acutely infected persons may play a disproportionate role in sustaining the epidemic.

Narrowing the window period before the virus can be detected has been a consistent goal in developing the development of new HIV tests and diagnostic algorithms. Most persons with acute infection develop clinical symptoms, but these are usually non-specific symptoms of a viral illness. Antibody tests cannot detect acute infection, but HIV RNA can be detected before antibodies develop. Nucleic acid amplification tests (NAAT) can identify the HIV RNA in acutely infected people who may still test negative or indeterminate by WB, IFA or other antibody tests. However, the specific role that NAAT should play in screening for and confirmation of HIV infection has yet to be defined.

Data on test performance are beginning to accumulate as US laboratories adopt new technologies that improve detection of acute infection, distinguish recent from longstanding infection and identify HIV-2 infections. More data are needed to evaluate the performance of recently-approved tests, such as NAAT. Outside the US, screening algorithms that utilize two different antibody tests to provide a high positive predictive value have been shown sufficient to initiate clinical staging with quantitative HIV RNA viral load and CD4 enumeration, without an intervening confirmatory test. More data on such an approach, using FDA-approved tests, are needed in the US. Given the diversity of goals of HIV testing, a menu of algorithms will likely be necessary to accomplish all the testing objectives.

In December 2007, CDC and APHL co-sponsored an HIV Diagnostics Conference⁸ to examine the algorithms drafted by the two HIV testing workgroups. Data presented at the conference substantiated some of the new alternative algorithms, and subsequent publications have added additional data, but more information is needed to validate the algorithms with all the assays currently available.

Note: In all algorithm schematics and descriptions, tests designated A1, A2, A3, etc., refer to different test products that ideally contain different antigens or that are based on a different test platform.

PROPOSED TESTING STRATEGIES FOR POINT OF CONTACT RAPID HIV TESTING

Point-of-Contact HIV Testing Algorithms – An Overview

Broadly defined, point-of-contact (POC) refers to HIV testing conducted on-site that can provide a waiting patient/ client with results in the same visit. In hospitals or similar clinical settings, POC may refer to HIV testing conducted at the patient bedside or in the examination room, and in some clinics/hospitals it may refer to HIV testing conducted in its stat laboratory. In community-based and outreach settings, POC refers to rapid HIV testing conducted in the presence of the client or in a separate area.

Most POC testing will be conducted with rapid HIV tests that are “waived” under the Clinical Laboratory Improvement Amendments (CLIA).⁹ CLIA categorized tests based on complexity. A test may receive a CLIA waiver if it uses direct, unprocessed specimens (such as whole blood or oral fluid) and is easy to perform with little chance of error. Waived tests may also be performed outside of traditional laboratories by individuals without formal laboratory training. To date, there are four rapid HIV tests available in the United States that are CLIA-waived.

Two additional rapid HIV tests have received FDA-approval. They may also be suitable for POC use; however, they are categorized by CLIA as moderately complex, a designation that requires laboratories to adhere to more stringent standards for personnel, quality assurance, and proficiency testing when using the tests. Some POC settings—such as a STD clinic—may be able to use a combination of waived and moderately complex rapid tests while the patient waits.

CDC’s quality assurance (QA) guidelines for rapid HIV testing¹⁰ state that rapid HIV tests are “used as screening tests to detect antibodies to HIV as part of multi-test algorithms to aid in the diagnosis of infection with HIV. Positive (reactive) rapid HIV test results are preliminary and must be followed up with an approved confirmatory test.” Non-reactive test results can be reported as negative. Reactive test results require supplemental testing with a FDA-approved test, such as a Western blot (WB), indirect immunofluorescence assay (IFA) or ribonucleic acid (RNA) test to confirm the diagnosis. POC Algorithm 1 reflects this current standard of practice, which was originally implemented when there was only one CLIA-waived rapid test. However, rapid HIV test package inserts¹¹ state that these tests are suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results. Since publication of the CDC quality assurance guidelines, several additional rapid HIV tests have been approved for the US market with this labeling.¹¹

POC Algorithms 2, 3 and 4 are proposed multi-test algorithms designed for statistical validation of rapid HIV test results. Multi-test algorithms have two objectives: to maximize the specificity and positive predictive value of POC test results and to facilitate same-day referral to care and treatment of persons with a likely HIV infection. As disease prevalence decreases, the percentage of reactive tests that are false-positives will increase (resulting in a lower positive predictive value) because there are fewer true-positives. It should be noted that none of the proposed POC strategies can improve the sensitivity of POC HIV screening; each strategy will be only as sensitive as the first test used in the algorithm. If a person has a recent high-risk of exposure, but

a non-reactive result on the initial rapid test, acute HIV infection testing should be considered (Laboratory Algorithm 4).

In clinical settings, multi-test algorithms provide additional information that can inform treatment decisions in a timely manner. In other settings, such as community-based and outreach venues, POC testing may be useful as a tool to facilitate rapid referral to care and treatment, particularly in those settings which serve transient or highly mobile populations, or those who may be unlikely to return for confirmatory test results.

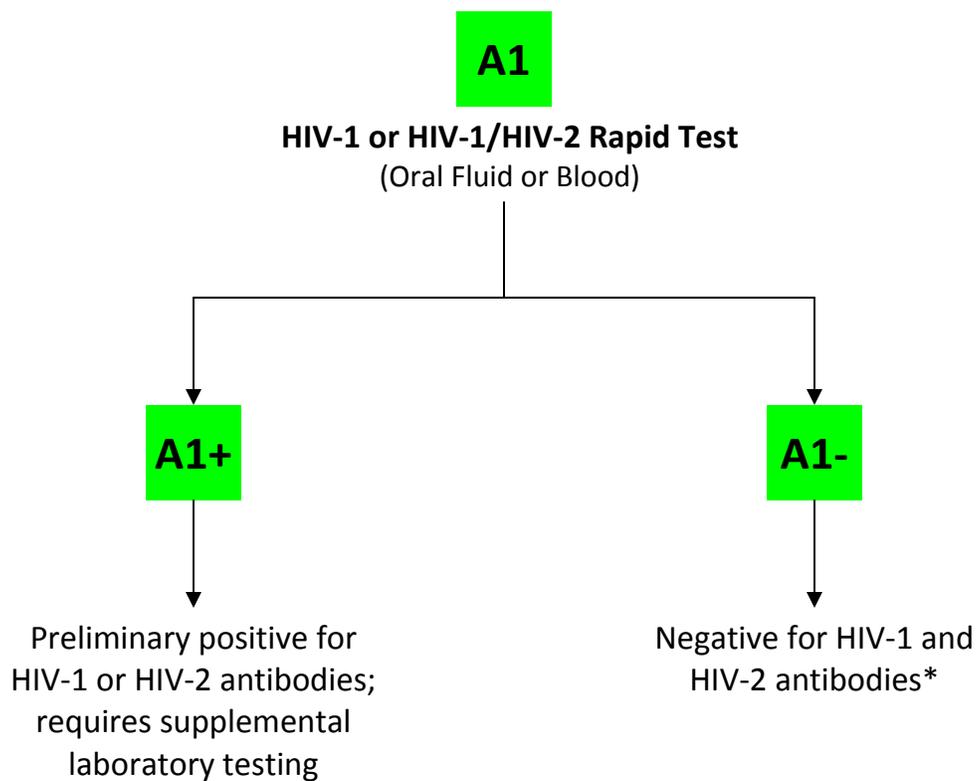
New York State and New Jersey Public Health testing programs have reported that high percentages of clients with reactive rapid test results (25.7%¹² and 25.8%¹³ respectively) failed to return for confirmatory test results. It is essential that agencies performing POC testing have well-established protocols for providing patients with the confirmatory test results, including follow-up procedures for clients who fail to return as scheduled. When using POC Algorithm 1, counselors, doctors and others involved should see all clients with preliminary positive results multiple times; these providers have the additional burden of trying to locate clients who fail to return as scheduled. Providers can use the information gained from the combinations of on-site tests used in POC Algorithms 2,3, and 4 to explain the meaning of the test results to the patient; to provide information and counseling that might help reduce any adverse consequences associated with false-positive results; or to facilitate a patient's engagement with needed care and prevention services. Perhaps most importantly, when multiple tests are used on-site, all of this can be done in one, efficient visit.

This report describes the development and supporting data of the testing components of the proposed algorithms. Additional work is needed to develop counseling messages and to integrate these algorithms into HIV program activities, but this is not the focus of this document.

Below are the description, rationale, and data to support each of four proposed POC testing algorithms. Unresolved issues for which more data are needed are highlighted. Refer to the key at the beginning of this document to clarify any acronyms or symbols.

Note: In all algorithm schematics and descriptions, tests designated A1, A2, A3, etc., refer to different test products that ideally contain different antigens or are based on a different test platform.

POC Algorithm 1 Single Rapid Test (A1) for HIV Screening



* If using an HIV-1 only rapid test, this result is negative **only** for HIV-1 antibodies.

Background

POC Algorithm 1, developed in 2004¹⁰ when the first CLIA-waived rapid HIV test received FDA approval, is currently used in most POC testing sites.¹⁴ In Algorithm 1, a single test is performed with an oral fluid or blood specimen.

Key Elements of the Design *(Refer to the POC Algorithm 1 schematic.)*

If the rapid test (A1) is reactive, then a “preliminary positive” result for the presence of HIV-1 and/or HIV-2 antibodies is reported. A specimen must be collected for supplemental laboratory testing to confirm the results. If the initial test (A1) is non-reactive, then a “negative” result for HIV-1 (or HIV-1 and HIV-2) antibodies is reported. Post-test information about the meaning of a negative test should be provided, and, if indicated, the patient should be counseled about the need for repeat testing or testing to rule out acute infection. Arrangements should be made to provide persons who have preliminary positive results with their confirmatory test results at the test site or at a referral site where HIV care is offered. Current guidelines recommend that persons with a reactive A1 test and a negative or indeterminate confirmatory test should be given a repeat confirmatory test one month after the initial discordant result.¹⁰

Available Data

Post-marketing surveillance has demonstrated that confirmatory WB or IFA results are sometimes negative or indeterminate in HIV-infected persons with reactive rapid tests.^{6,14} In the largest report of data on this algorithm, Wesolowski et al., found that, of 167,371 rapid tests conducted in 16 health departments between July 2004 and December 2005, 2589 (1.6%) were reactive and required laboratory confirmation. Of these, 2417 (93%) had positive WB/IFA and 172 (7%) had negative or indeterminate WB/IFA. Of the 172 cases unconfirmed by WB/IFA, 83 (48%) people failed to have repeat confirmatory testing performed. Of the 89/172 (52%) persons with a repeat confirmatory test: 17 (19%) were HIV-infected, including three with indeterminate WB and positive NAAT; 72 (81%) were uninfected, including 12 with repeat indeterminate WB.¹⁴ Data from the New York State Department of Health (NYS)¹⁵ document that a supplemental RNA test resolved discordant confirmatory test results for 76 rapid test reactive specimens that tested negative or indeterminate by Western blot. Of the 18 rapid test reactive, but WB-indeterminate specimens, 14 were NAAT-positive, three were NAAT-negative, and one was invalid. Of 58 rapid test reactive specimens with negative WBs, two were NAAT-positive and considered likely early infections, 54 were NAAT-negative, and two NAAT tests were invalid. Other data indicate that the sensitivities of rapid tests differ during acute infection and may be less sensitive shortly after development of an antibody response when compared to conventional immunoassays.^{16,17}

Summary

Benefits

- This algorithm efficiently identifies most uninfected individuals.
- It identifies those likely to be infected and in need of further testing.
- It is suitable for settings where quality assurance of multiple products is not feasible.

Drawbacks

- Some preliminary results will be false-positive, causing distress for clients and providers.

- Some persons with early HIV infection may receive false-negative results.
- Entry to care/treatment and partner notification may not occur until after confirmatory testing.
- Clients may not receive confirmatory test results if appropriate follow-up procedures are not in place, especially for transient populations.

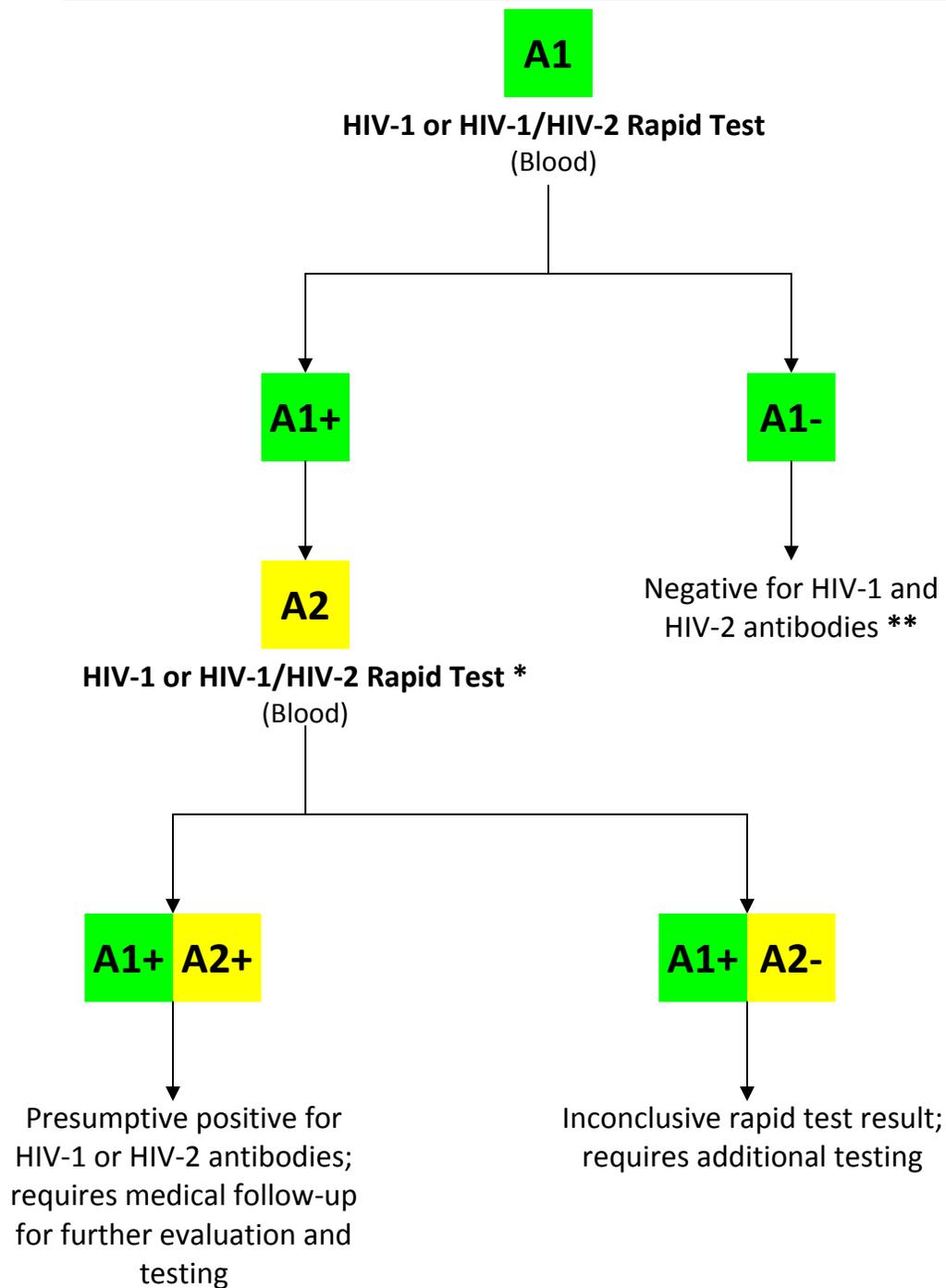
Additional Data Needed to Substantiate and Refine the Algorithm

Because this algorithm is used currently by nearly all POC testing sites, most of the data needed to substantiate this algorithm relate to the performance of newly-approved tests. Examples of additional data that would further validate POC Algorithm 1 include:

- Performance (sensitivity and specificity) of new tests
- Rates of false-negative screening tests (based on results of acute infection screening or follow-up testing)
- Rates of false-negative or indeterminate Western blot results among infected persons, based on NAAT or subsequent follow-up

POC Algorithm 2

Two Rapid Tests (A1/A2) Performed in Sequence on Blood



* Test must be from a different manufacturer.

** If using an HIV-1 only rapid test, this result is negative **only** for HIV-1 antibodies.

Background

In POC Algorithm 2, two different rapid test products are used sequentially, both on blood specimens, to improve the positive predictive value of POC testing. It assumes that the first (A1) rapid test will have equivalent or better sensitivity than the second (A2) test. The goal of this strategy is to differentiate persons who are likely to be infected (both A1 and A2 reactive) from those who most likely had a false-reactive screening test result (reactive A1, negative A2).

Key Elements of the Design *(Refer to the POC Algorithm 2 schematic.)*

If the A1 rapid test is non-reactive for HIV-1 and/or HIV-2 antibodies, a “negative” result for HIV-1 and/or HIV-2 antibodies is reported. If the A1 test is reactive, a second test (A2) is immediately performed. The A2 kit reagents must be produced by a different manufacturer from those used in the A1 test. If both A1 and A2 are reactive, the result is reported as: “Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If A1 is reactive and A2 is non-reactive, the result is reported as: “Inconclusive rapid test result; requires additional testing.”

Algorithm 2, through the use of two rapid tests, provides additional information to patients who are reactive with both tests: most will be truly infected and can be referred to medical care at the same visit. Those who have discordant test results are more likely to have had a false-positive result on the initial rapid test. This algorithm will deliver more accurate information, but confirmatory tests results must be delivered (at a later date) to resolve the discordance.

Available Data

There is limited clinical data from the US for the sequential use of two different rapid tests with whole blood specimens. A study by Houkoos et. al, describes an emergency room population in which 1539 patients were tested using a whole blood assay.¹⁸ Of the five reactive A1 tests (0.3%), four were also reactive on the A2 test. All four A1+/A2+ specimens were confirmed Western blot positive. For the one A1+/A2- result, the Western blot was negative.

Data collected during prospective evaluations of algorithms that use three different HIV test products, as well as from retrospective evaluations using stored specimens, are also available to help evaluate Algorithm 2. The New Jersey Department of Public Health (NJDPH) Laboratory presented data from 363 individuals tested during 2003-2005. These individuals were reactive on an A1 blood test in the field and submitted specimens to the NJDPH laboratory for confirmation.¹³ The specimens were subjected to Western blot testing, as well as testing with additional rapid blood tests (Unigold, Reveal and Multispot). Of the 363 specimens, 355 (97.8%) were reactive by the screening test (A1), all follow-up rapid tests (A2) and WB. The remaining eight (2.2%) A1+ specimens were negative by the follow-up rapid tests and WB. Notably, when the A1 test was repeated subsequently on the eight false-positive specimens, they all remained reactive, providing evidence that supports the use of a different rapid antibody test for A2

A presentation from CDC included data from a prospective evaluation of all FDA-approved rapid tests, in which the performance of the various POC algorithms was modeled.¹⁹ The evaluation showed that a two-test algorithm was able to distinguish true-positive from false-positive antibody screening tests in nearly all instances. Fifty-four potential two-test combinations were evaluated, using between 156 and 230 reactive A1 tests out of a total sample of 4518 specimens. Three specimens were false-positive on at least two tests. Modeling of the

variability in two-test algorithm performance suggested that adding an A2 test at POC would improve the median specificity from 99.87% (bootstrap confidence intervals (CI): 99.75-99.98) for the A1 test to 100% (CI: 99.97-100) for all potential combinations of A1/A2.

When prevalence was varied and test performance characteristics were held constant, modeling showed potential differences in the outcomes of A1+/A2- tests among various test kit combinations. In the most extreme examples of A1 test performance, with 4.8% HIV infection prevalence observed in this population and 99.23% specificity of the A1 test, of 255 reactive A1 tests, 219 infected persons would have been resolved correctly as A1+/A2+, but one (0.5%) A1+/A2+ result would be false-positive. The other 35 (14%) A1+/A2- specimens would have been sent to a laboratory for subsequent confirmatory testing, of which two were from infected patients; the other 33 were false-positive A1 test results. However, if the same A1 test was used in a setting with an HIV infection prevalence of 0.1%, then, on average, five specimens would have been A1+/A2+ and all five would have represented true infections. At 0.1% prevalence, on average, 33 (87%) specimens would be A1+/A2- and require subsequent laboratory resolution, but in this instance, all would have represented false-positive A1 tests. In other words, as HIV prevalence decreases, the likelihood of observing a false-negative A2 test also decreases. The number of A1+/A2- tests that require laboratory resolution remains approximately the same regardless of prevalence; but as prevalence decreases, these represent an increasing percentage of all reactive A1 tests that cannot be resolved at POC.

Summary

Benefits

- This algorithm efficiently identifies most uninfected individuals.
- It improves the positive predictive value of POC testing when two tests are reactive.
- Individuals who test positive on two rapid tests can be informed they are very likely to be HIV-infected and can efficiently be referred for further care and treatment.
- It can be used by non-laboratorians, provided that proper and appropriate quality assurance is in place.

Drawbacks

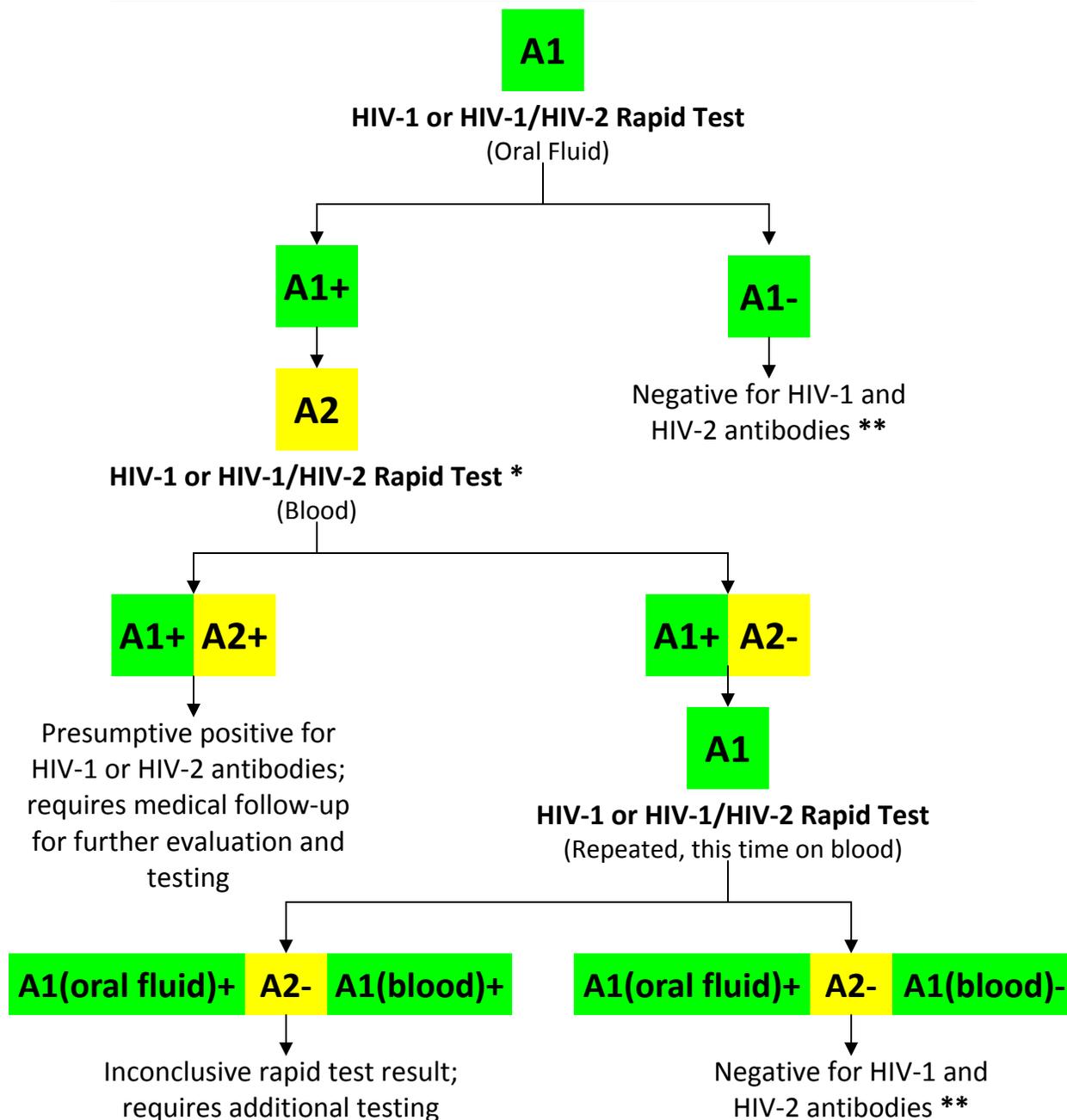
- The two-test algorithm still produces a small number of false-positive results, causing distress for clients and providers.
- Some persons with early HIV infection may receive false-negative results.
- It may be more challenging to maintain inventory, technical proficiency and overall quality assurance for multiple rapid tests.

Additional Data Needed to Substantiate and Refine the Algorithm

- Discern the number of false-negative and false-positive results of the possible combinations of POC rapid tests.
- Data for different specific sequences of two different POC rapid tests performed prospectively in diverse settings that represent both high and low HIV prevalence
- Determine the rates of false-negative results for different tests used as A1 in POC settings.
- Assess the effectiveness of same-day referral to clinical care for A1+/A2+ patients, in comparison to patients receiving results from just a single rapid test (Algorithm 1).

- Evaluate whether clients who receive A1+/A2- test results at the initial visit, but are actually uninfected, are more likely to return for confirmatory laboratory results than clients receiving reactive results from a single rapid test (Algorithm 1).

POC Algorithm 3
Two Rapid Tests (A1 Oral Fluid/A2 Blood) Performed in
Sequence; If A2 Is Negative, A1 Is Repeated on Blood.
 (A1 and A2 must be different rapid tests) †



* Test must be from a different manufacturer.

** If using an HIV-1 only rapid test, this result is negative **only** for HIV-1 antibodies.

† This algorithm may only be used when the same test is available for both oral fluid and blood.

Background

POC Algorithm 3 is designed to improve the positive predictive value of rapid HIV testing, using an oral fluid specimen. In this algorithm, a reactive oral fluid test (A1) is followed by a different test (A2) performed on a blood specimen.

Key Elements of the Design *(Refer to the POC Algorithm 3 schematic.)*

Algorithm 3 is a special case of POC Algorithm 2, in which A1 is performed on oral fluid, A2 on a blood specimen, and A1 can be repeated on a blood specimen. It is designed to address the decreased specificity observed with oral fluid screening. As in POC Algorithm 2, if both A1 and A2 are reactive, the result is reported as: “Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.” If the A1 test is reactive with both oral fluid and blood, but the different A2 test is non-reactive with blood, these results may represent either A1 false-positives with two specimen types, or an A2 false-negative. In this scenario, the result should be reported as: “Inconclusive rapid test result; requires additional testing.” If the A1 test on an oral fluid specimen is reactive, but both the A2 and A1 tests are non-reactive with blood, the result should be reported as: “Negative for HIV-1 and HIV-2 antibodies.” In this case, the oral fluid test is considered a false-positive result and the blood test results are considered true-negative results.

Available Data

At the 2007 HIV Diagnostics Conference, there were presentations from three different studies, each using slightly different prospective testing strategies to maximize the positive predictive value of oral fluid testing. One laboratory validation of Algorithm 3 was also presented.

The New York City Health Department reported its experiences (later updated in the June 2008 MMWR)²⁰ with a two-specimen-type algorithm, in which a reactive oral fluid test was followed with a rapid test from the same manufacturer on a blood specimen. Of 1,194 reactive oral fluid rapid tests, 840/850 (98.8%) reactive with both oral fluid and whole blood were confirmed positive by subsequent Western blot (WB). Of the 344 that were negative with whole blood, 343 (99.9%) were negative by Western blot.

Two presentations from the San Francisco Department of Public Health^{21, 22} reported experiences of developing and implementing strategies using an initial oral fluid rapid test that, if reactive, was followed by two blood rapid tests, in high HIV prevalence and high HIV incidence settings. In this study, of 30 A1+ specimens, 22 were A1+/A2+, and then confirmed WB-positive. The eight A1+/A2- specimens were A1- with blood and WB-negative.

In another study, researchers from Highland Hospital in Oakland, CA, described their strategy: following a reactive A1 oral fluid test, blood specimens were tested with two additional tests (A2) and the A1 test was repeated on blood.²³ Of 52 A1+ oral fluid tests, 38 were A2+ on both additional tests, and all were confirmed positive by WB. Twelve of the remaining 13 were negative on both A2 tests and the repeated A1 rapid test performed on blood as well as WB; one specimen was reactive on one of two A2 tests and on A1 performed on blood, and indeterminate by WB. After several follow-up appointments and retesting, this client was determined to be HIV-negative.

Finally, investigators from the New Jersey Rapid HIV testing program reported on the follow-up testing of specimens which screened A1+ on an oral fluid rapid test, but failed to confirm as Western blot positive (n=156) between 2006 and 2007.¹³ In this retrospective laboratory evaluation, none of the false-positive specimens were reactive when retested with different rapid tests, although one (0.6%) specimen was reactive on the same rapid test when it was performed on blood. None of the 892 reactive oral fluid screening tests that were confirmed positive by WB during this time were retested with other rapid tests.

In these presentations, the evidence showed that following a reactive oral fluid screening test with at least one additional rapid test on a blood specimen improved the positive predictive value of the testing. The New York City Health Department presentation contained prospectively collected data that showed 2.8% (10/353) of the false-positive oral fluid rapid tests were also false-positive with the same rapid test on blood. Although fewer false positive oral fluid test results were identified in San Francisco, Oakland and New Jersey (N=177 combined), two (1.1%) were false-positive when A1 was repeated on blood, but none was false-positive on a different whole-blood rapid test. Combined, these data suggest that using the same rapid test on a blood specimen following a reactive rapid test on an oral fluid specimen resolved most (518/530 or 97.7%), but not all, false-positive results from an initial rapid oral fluid test. These data support the recommendations contained in POC Algorithm 3, which include using a second, different rapid test on a blood specimen between two rapid tests of the same manufacturer, in order to eliminate as many false-positives as possible.

Unlike POC Algorithm 2, in which only retrospective analyses of the algorithm have been developed, algorithms for testing after a reactive oral fluid rapid test have been prospectively evaluated in several different US settings. Although the total number of A1 tests performed is large (more than 130,000 tests in NYC; more than 77,000 in New Jersey; and more than 12,000 in Oakland's emergency department), there were only a limited number of positive test results (either true- or false-positive), making it difficult to validate the relative performance of the algorithms. However, these field evaluations suggest that following a reactive oral fluid test with either a different or the same rapid test on whole blood can substantially increase the positive predictive value.

Summary

Benefits

- This algorithm efficiently identifies most uninfected individuals.
- It improves the positive predictive value of reactive oral fluid test results at the POC.
- Individuals who test positive on both an oral fluid and a different blood rapid test can be informed that they are likely to be HIV-infected and be referred efficiently to further care and treatment.
- Individuals who test positive on an oral fluid test, but test negative on two blood tests, can be informed that they are likely to be uninfected.
- It can be used by non-laboratorians, provided that proper and appropriate quality assurance is in place.

Drawbacks

- Some persons with early HIV infection may receive false-negative results.

- It may be more challenging to maintain inventory, technical proficiency and overall quality assurance for multiple rapid tests in some settings.

Additional Data Needed to Substantiate and Refine the Algorithm

- More data are needed on the performance of specific A2 tests after a reactive A1.
- More prospective evaluations of the rate of occurrence of false-positive results for the same test conducted at the same visit on different specimen types.
- Specific studies of the reasons for sporadic increases in the rates of false-positive oral fluid tests would also help inform the use of this algorithm relative to Algorithms 2 or 4.
- Additional comparative evaluations of algorithms that use a third different (A3) test, instead of repeating A1 on blood, to quantify the differences in implementation and performance of two- versus three-test algorithms that begin with an oral fluid test.
- Additional data on new, but not yet FDA-approved, oral fluid HIV tests are warranted when these tests become widely available.
- Evaluation of whether clients who are actually uninfected are more likely to return for laboratory confirmatory results after receiving A1+/A2-/A1+ test results at the initial visit, compared with receipt of reactive results from a single rapid test (Algorithm 1).

Background

POC Algorithm 4 is common in settings with limited access to laboratory tests and uses three different rapid tests to provide a greater positive predictive value for the test results in low prevalence settings. This strategy has been proposed as a way to confirm results at POC, but requires further validation before it can be implemented. This three-rapid-test strategy calls for the use of a blood specimen (A1) initially; if that test is reactive, a second test from a different manufacturer (A2) is conducted on a blood specimen. If A2 is negative, a third test (A3) from a third manufacturer would be performed, also on a blood specimen. The test most sensitive for early infections should be used for A1.

Key Elements of the Design *(Refer to the POC Algorithm 4 schematic.)*

If A1 is reactive, A2 should be performed, also on a blood specimen. If A2 is reactive, the result is reported as: “Presumptive positive for HIV-1 and/or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.”

If A2 is negative, then a blood specimen should be tested again using a third different test (A3). If the A3 test is reactive, then, evaluated in conjunction with a reactive A1 test and a non-reactive A2 test, the result should be reported as: “Presumptive positive for HIV-1 and/or HIV-2 antibodies; requires medical follow-up for further evaluation and testing.”

If A3 is negative, and is in conjunction with a reactive A1 test and a negative A2 test, then it is suggested currently that the result be reported as: “Inconclusive rapid test result; requires additional testing.”

Available Data

Modeling studies performed by CDC using data from a prospective evaluation of all FDA-approved rapid tests illustrated the interplay between the performance of a given sequence of tests as HIV prevalence changes.¹⁹ Assumptions about algorithm performance were held constant: the same number of false-positive results will be generated when a given strategy is performed on a known number of HIV-negative specimens, regardless of prevalence. As prevalence decreases, the percentage of all reactive tests that are false-positives will increase (lower positive predictive value) because there are fewer true-positives. These data illustrate the value of Algorithm 4 for low prevalence settings, where the majority of reactive screening tests are false-positive results that can be resolved with a POC algorithm.

Data from the San Francisco Public Health Laboratory¹⁶ and the Seattle King County Department of Public Health¹⁷ suggest that some rapid tests may be slightly more sensitive for early infection than others, creating the possibility that a reactive rapid blood test that is followed by two additional non-reactive rapid tests cannot rule out the potential for an early HIV infection at the POC. The San Francisco Public Health Laboratory provided data showing rapid test performance on 42 persons identified through their acute infection screening program.¹⁶ On the initial specimens that were NAAT-positive and flagged as possible acute infections, Unigold detected 11/42, Multispot 7/42, Statpak 1/42 and Oraquick 1/42 (the same one detected by Statpak). The data presented by the Seattle King County Department of Public Health suggested that Oraquick was false negative in 26/149 (17%) of cases with early HIV infection (10 EIA-reactive, and 16 EIA-negative, NAAT-reactive) among frequently-tested gay men at the Seattle men’s health clinic.¹⁷

These data show the sensitivity limitations of CLIA-waived rapid tests when used early in infection, when antibody response is still developing, and suggest caution when interpreting, for example, a reactive Unigold result followed by non-reactive Oraquick and Statpak results using Algorithm 4. Such test results are likely to indicate that the initial (A1) test result was false-positive. However, due to the absence of sufficient data, a recommendation that non-reactive A2 and A3 results may supersede a reactive A1 result, leading to an overall HIV negative result at the POC, cannot be made at this time. Studies of POC Algorithm 4 in high incidence settings are ongoing, and these results will be considered when they are available.

Summary

Benefits

- This algorithm efficiently identifies most uninfected individuals.
- It improves the positive predictive value of reactive rapid test results at the POC.
- Individuals who test positive on two of three blood rapid tests can be informed that they are likely to be HIV-infected and be referred efficiently to care and treatment.
- Individuals who test positive on only one of three blood rapid tests can be informed that they are not likely to be infected, although additional testing is recommended.
- It can be used by non-laboratorians, provided that proper and appropriate quality assurance is in place.

Drawbacks

- Some persons with early HIV infection may receive false-negative results.
- Data are limited on the algorithm's performance with different sequences of specific tests.
- It may be more challenging to maintain inventory, technical proficiency and overall quality assurance for multiple rapid tests in some settings.

Additional Data Needed to Substantiate and Refine the Algorithm

- Data for different specific sequences of three different POC rapid tests performed prospectively in diverse settings that represent both high and low HIV prevalence.
- Rates of false-negative test results from different tests used as the initial (A1) test.
- An evaluation of the effectiveness of same day referral for getting A1+/A2+ or A1+/A2-/A3+ patients into clinical care, compared with receipt of results from just a single rapid test (Algorithm 1).
- Evaluation of whether clients, who are actually uninfected, are more likely to return for laboratory confirmatory results after receiving A1+/A2-/A3- test results at the initial visit, compared with those receiving reactive results from a single rapid test (Algorithm 1).

PROPOSED TESTING STRATEGIES FOR LABORATORY HIV TESTING

Laboratory Testing Algorithms: An Overview

In general, laboratory testing employs assays categorized by CLIA as moderate or high complexity. Depending on the size of the laboratory and specific application, some laboratories may use rapid HIV tests (categorized under CLIA as moderately complex when used with serum or plasma) as one of the component assays of the laboratory testing algorithms. In these algorithms, the term “immunoassay” is used to encompass traditional enzyme immunoassays (EIA), chemiluminescent assays (CIA) and single-use rapid immunoassays.

Early laboratory-based immunoassays, and most rapid HIV tests, are indirect EIAs. HIV antigen is applied to the solid phase in the test kit. If antibodies are present, the antigen–antibody complex is detected using an anti-human IgG antibody conjugated to an enzyme. A substrate is then added from which the bound enzyme generates a colorimetric reaction if anti-HIV IgG is present.

Increasingly, laboratory-based immunoassays and some rapid tests incorporate the “antigen sandwich” technique. Viral antigen is applied to the solid phase, but detection is accomplished with enzymes or esters conjugated to HIV antigen (instead of to anti-human IgG). HIV antibody in serum thus binds to HIV antigen(s) in the solid phase and to the HIV antigen in the conjugate. This “antigen sandwich” detects any class of anti-HIV antibody, including IgM, and is subject to lower background optical densities, which improves analytic sensitivity. Accumulating evidence indicates that, in early infection, sandwich immunoassays demonstrate greater sensitivity than older EIAs, Western blot (WB) and indirect immunofluorescence (IFA) confirmatory assays.

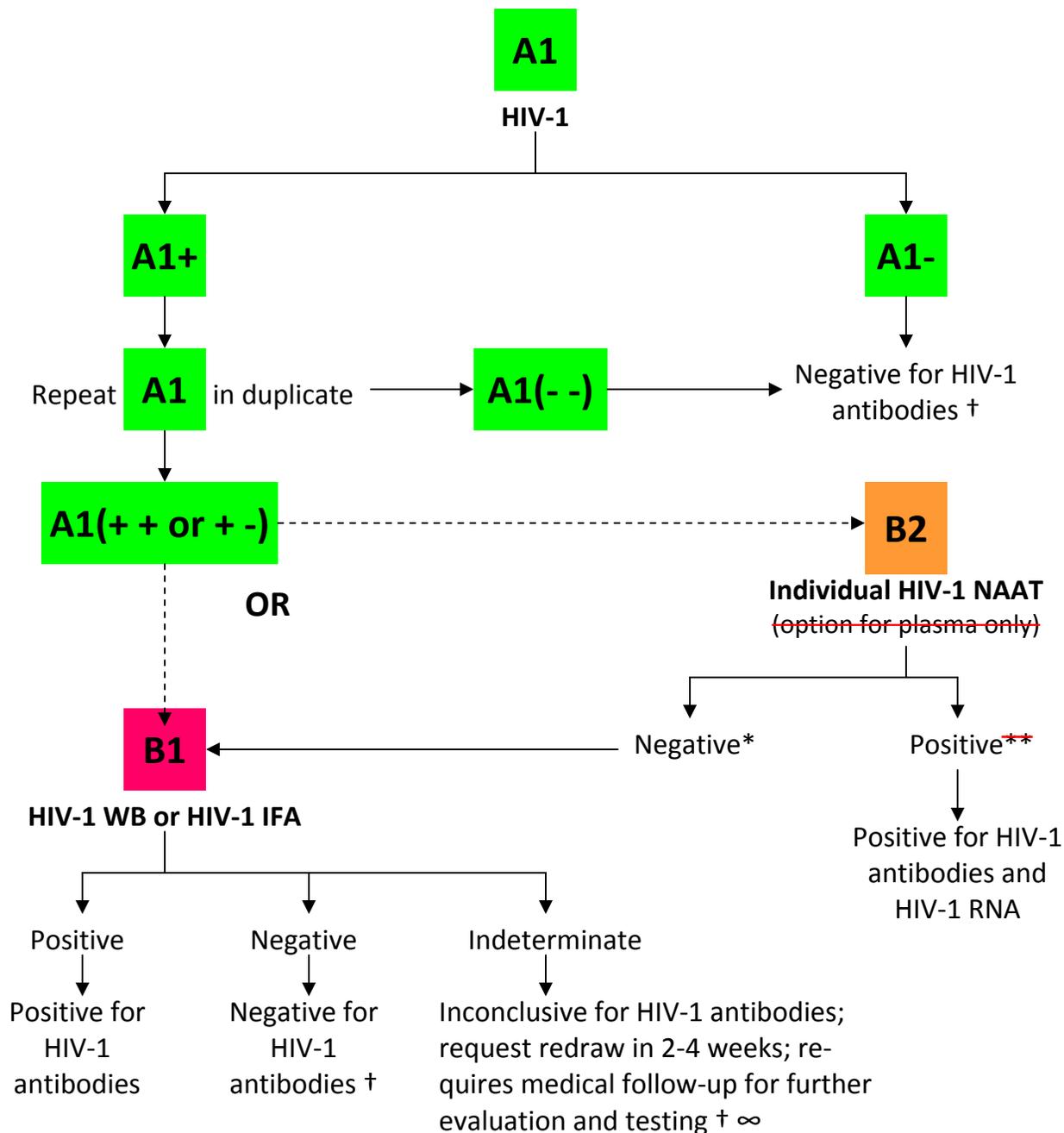
In addition, molecular techniques, such as nucleic acid amplification testing (NAAT), now offer alternatives for even earlier detection of infection. The proposed laboratory-based testing algorithms attempt to incorporate these considerations, and offer options (indicated by dotted lines in the algorithm schematics) that may be selected for use depending on the laboratory’s capabilities and clinical context.

Laboratory testing may serve several functions: screening and confirmatory testing of specimens submitted for initial evaluation, and confirmatory testing of specimens submitted after reactive laboratory screening tests or reactive results from a POC algorithm. In the latter case, the POC algorithm result becomes the result of the initial (A1) screening assay. If laboratories choose to perform an A1 screening assay, current protocols require confirmatory testing with a supplemental test, regardless of the in-lab result.^{6, 10}

Below are the description, rationale, and data to support each of five proposed laboratory-based testing algorithms. Unresolved issues for which more data are needed are highlighted. (See the key at the beginning of this document for clarification of acronyms or symbols.)

Laboratory Algorithm 1

HIV-1 Only Immunoassay, With Supplemental NAAT Option



* HIV-1 RNA is not detected; however, WB or IFA should be performed to confirm the absence of HIV-1 antibodies.

~~** It may be necessary to repeat a positive NAAT for confirmation.~~

† If a window period infection is suspected based on risk assessment or discordant testing, refer to Acute HIV Infection Testing Laboratory Algorithm 4.

∞ If HIV-2 infection is suspected, refer to HIV-2 Testing Laboratory Algorithm 5.

Laboratory Algorithm 1

HIV-1 Only Immunoassay, with Supplemental NAAT Option

Background

This laboratory-based algorithm reflects most closely the original CDC/APHL algorithm established in 1989, a stand-alone HIV-1 immunoassay as the (A1) screening test followed by a (B1) supplemental test, either WB or IFA.¹ In Laboratory Algorithm 1, that original algorithm has been modified to include an optional pathway for NAAT in the confirmatory process; yet, it maintains its initial structure for laboratories that test dried blood spot specimens or consider it adequate for their test population. However, currently available HIV-1 immunoassays are indirect EIAs, and although those incorporating whole viral lysate demonstrate some cross-reactivity with HIV-2 antigens, Algorithm 1 may not be adequate if HIV-2 infections are suspected or if there is an emphasis on detecting acute and early HIV-1 infections.

In the fall of 2006 the first qualitative HIV-1 RNA NAAT was approved for the diagnosis of acute HIV-1 infections (see Laboratory Algorithm 4 for more information on NAAT use) and for confirming HIV-1 infection when tests for antibodies are repeatedly-reactive. The advantages of the NAAT option as a supplemental test for confirmation in Algorithm 1 are its less subjective result interpretation and its potential to decrease the number of indeterminate HIV-1 antibody laboratory results.

Key Elements of the Design *(Refer to the Laboratory Algorithm 1 schematic.)*

Algorithm 1 allows laboratories to test immunoassay-reactive specimens for evidence of HIV infection as they have always done, with WB or IFA (B1), or incorporate NAAT (B2) for confirmation. After a repeatedly-reactive A1, individual NAAT can be used as the B2 supplemental test for confirmation; however, laboratories should note that a negative NAAT result does not rule out infection. If the individual NAAT (B2) is negative following a repeatedly-reactive A1, follow-up testing with WB or IFA (B1) is still required. If the NAAT (B2) is not performed initially, it may then be used in an attempt to resolve indeterminate or negative WB or IFA (B1) test results for persons with possible recent HIV-1 infection. ~~At present, the FDA approved NAAT assay requires a plasma specimen.~~ Additionally, Algorithm 1 does not specifically screen for or confirm HIV-2 infection.

Available Data

Though only approved recently for use in diagnostic testing, NAAT has been used for several years as a supplemental donor screening assay in US blood centers. Data provided by the American Red Cross and Blood Systems Laboratory indicated that NAAT had 100% concordance with 317 WB-positive results and resolved an additional 16 false-positive WBs as negative. In addition, 98.6% (3,388/3,434) of indeterminate WB interpretations in uninfected donors were resolved.²⁴

At the 2007 HIV Diagnostic Conference, data from New York State showed that NAAT for HIV-1 RNA resolved 92% (94/102) of cases that were HIV-1/2 EIA repeat-reactive (RR) and WB negative or indeterminate as actually HIV-1 RNA negative.¹⁵ New York State did not present data on the ability of RNA testing to identify EIA-RR, WB-positive specimens correctly.

From May 2006 to February 2008, Florida performed individual NAAT on 641 EIA-RR/WB-

positive specimens; all of these had detectable HIV-1 RNA. Additionally, subsequent seroconversion was documented for three of four EIA-RR/WB-indeterminate/NAAT-positive specimens, and for two of four EIA-RR/WB-negative/NAAT-positive specimens. Thus, five people were informed earlier of HIV infection based on the NAAT results and without need of a follow-up specimen. However, NAAT results were false-positive in three persons with EIA RR and a negative or indeterminate WB.²⁵

In a recent article by Owen, et al., the sensitivity of three different NAAT assays ranged from 93-97% on 621 EIA-RR/WB-positive specimens, but it is not known whether any of these specimens came from persons treated with antiretrovirals.²⁶ Thus, data support the use of NAAT as a supplemental test for confirming EIA-RR specimens, especially during early infection; but supplemental serologic testing is required for EIA-RR, NAAT-negative specimens.

Recent data from a large metropolitan rapid test study included comparisons of WB and NAAT for confirmation of reactive rapid test results. In this study, 273/280 (97.5%) prospectively identified rapid test and WB-positive specimens were also NAAT-positive,²⁷ but seven WB-positive specimens were NAAT-negative. In another study, New York State resolved 14/18 (78%) rapid test-reactive/WB-indeterminate results as RNA-positive; three were RNA-negative; and one was invalid. Additionally, 54/58 (93%) samples that were rapid test-reactive/WB-negative were resolved as RNA-negative; two were RNA-positive and two were invalid.¹⁵

Summary

Benefits

- FDA-approved assays are available for use as A1 and B1 with alternative specimens such as dried blood spots.
- Use of NAAT (B2) can effectively confirm most reactive A1 immunoassay tests.
- Use of NAAT (B2) reduces WB/ IFA indeterminate results in specimens from persons with reactive A1, especially during early HIV infection.
- Offering options B1 or B2 for confirmation accommodates laboratories that are not equipped to perform NAAT.

Drawbacks

- Available HIV-1 assays are less sensitive during early infection than current HIV-1/2 combination assays and do not reliably detect HIV-2 antibodies.
- A1-RR, B2-negative specimens require further testing with B1.
- HIV-1 only assays may not remain available commercially.
- ~~Currently, B2 (NAAT) requires plasma specimens.~~
- Using NAAT assays that are not FDA-approved for diagnosis (e.g., quantitative viral load assays) requires validation by individual laboratories.

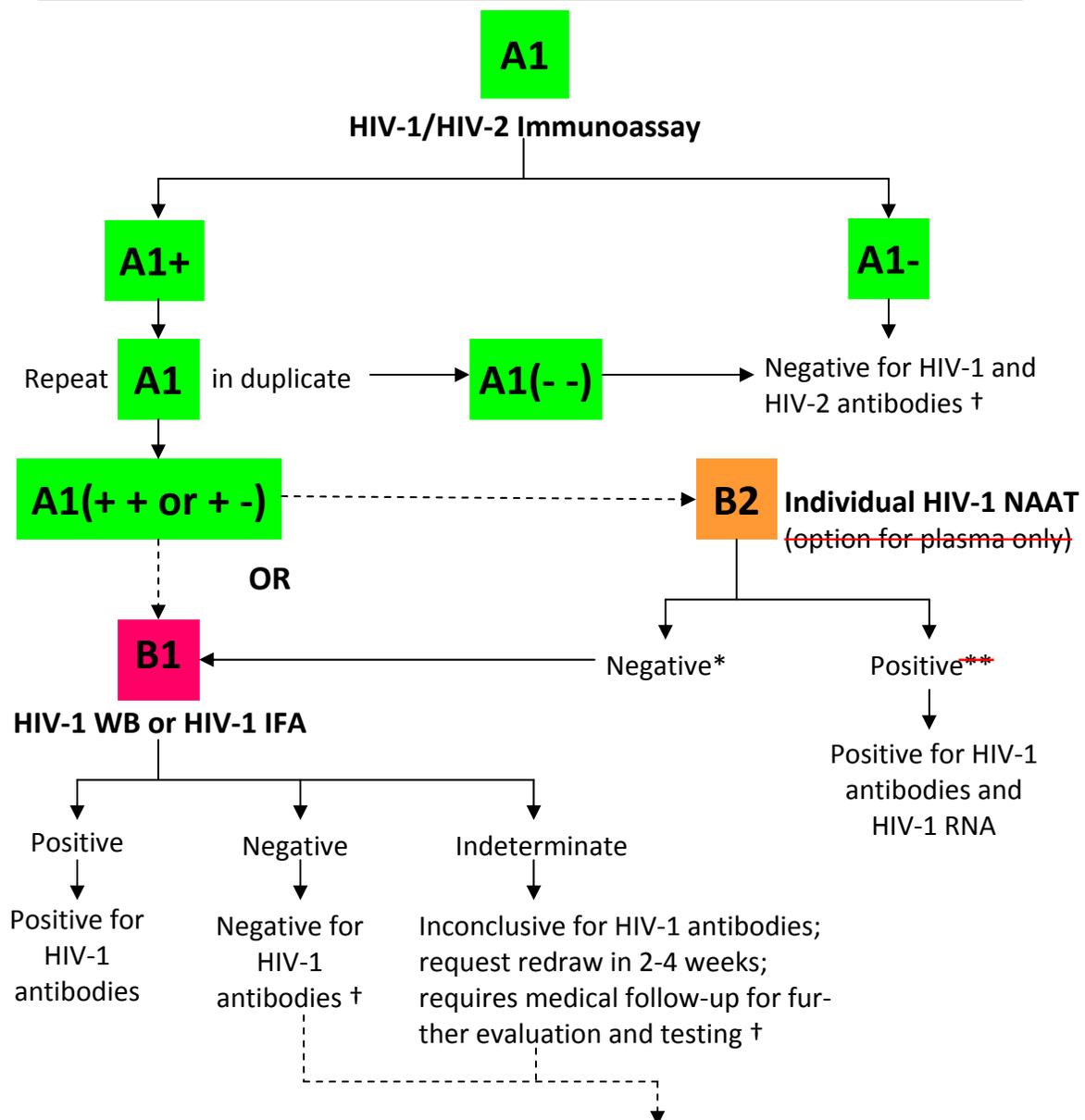
Additional Data Needed to Substantiate and Refine the Algorithm

- Number and percentage of initially-reactive A1 results that are not repeatedly reactive to determine if it is necessary to repeat a reactive A1.
- Signal-to-cutoff ratios (S/CO) of initially-reactive A1 and S/CO for repeat testing to validate the utility of this additional information for interpretation.

- Additional data to evaluate NAAT as supplemental test. This includes comparison data between tests that detect HIV-1 RNA or DNA (FDA licensed and unlicensed) supplemental antibody tests, or with follow-up for seroconversion.
- Performance of NAAT as a confirmatory test after a reactive rapid test
- Number and percentage of false-negative NAAT results in specimens confirmed positive by a different supplemental HIV-1 NAAT, supplemental antibody test or medical evaluation and follow-up in antiretroviral-naïve patients
- ~~Performance data on qualitative NAAT used with serum instead of plasma to determine whether serum produces acceptable results for confirmation~~
- Number and percentage of false-positive HIV-1 supplemental antibody tests, as determined by follow-up
- Number and percentage of false-negative or indeterminate HIV-1 supplemental antibody tests as determined by NAAT on original specimens and medical evaluation or follow-up for seroconversion
- Logistics of NAAT implementation (specimen type, specimen handling/storage, automation, etc) and the impact on reporting turn-around time and cost

Laboratory Algorithm 2

HIV-1/HIV-2 Immunoassay, With Supplemental NAAT Option



HIV-2 Laboratory Algorithm 5, if one or more of the following apply:

- 1) Indicated by local HIV-2 prevalence
- 2) Indicated by travel or risk history
- 3) Indicated by clinical presentation
- 4) Indeterminate WB pattern

* HIV-1 RNA is not detected; however, WB or IFA should be performed to confirm the absence of HIV-1 antibodies.

~~*** It may be necessary to repeat a positive NAAT for confirmation.~~

† If a window period infection is suspected based on risk assessment or discordant testing, refer to Acute HIV Infection Testing Laboratory Algorithm 4.

Laboratory Algorithm 2

HIV-1/HIV-2 Immunoassay, with Supplemental NAAT Option

Background

The inclusion of antigens designed to detect the HIV-2 strain of the virus is becoming increasingly common in FDA-approved HIV diagnostic tests.⁷ Because global migration and international travel increase the potential distribution of HIV-2, it is important to have an algorithm capable of detecting infected patients. Many of the HIV-1/2 immunoassays also detect both IgM and IgG antibodies. Laboratory Algorithm 2 incorporates the use of these more sensitive screening assays capable of detecting both HIV-1 and HIV-2 antibodies, and the option of HIV-1 NAAT for confirmation.²⁶

In the fall of 2006 the first qualitative HIV-1 RNA NAAT was approved for use in the diagnosis of acute HIV-1 infections (see Algorithm 4 for more information on NAAT) and for confirming HIV-1 infection after antibody tests are repeatedly-reactive. The primary advantage of the NAAT option in Algorithm 2 as a supplemental test for confirmation is its less subjective result interpretations and its potential to decrease the number of inconclusive HIV-1 antibody results. HIV antibody tests that detect both HIV-1 and HIV-2 have already been shown to be more sensitive to early HIV infection than the traditional confirmatory tests, HIV-1 Western blot and IFA. However, HIV-1 RNA NAAT does not react with HIV-2 nucleic acids, unlike the cross-reactivity that HIV-1 Western blot and IFA frequently display with HIV-2 antigens. Algorithm 2 has the potential to identify persons infected recently with HIV-1 who might have been reported as negative or indeterminate after WB or IFA, and deals more explicitly with the detection of HIV-2 infections.

Key Elements of the Design *(Refer to the Laboratory Algorithm 2 schematic.)*

Algorithm 2 has a very similar structure to Laboratory Algorithm 1. Algorithm 2 offers the option of using B1 (HIV-1 WB or IFA) or B2 (HIV-1 NAAT) for a supplemental test on an A1 immunoassay repeatedly-reactive (RR) specimen. Algorithm 2 has the potential to detect more infections in the early stages of disease, but a negative NAAT result does not rule out infection. As in Algorithm 1, if B2 (NAAT) is negative following a repeatedly-reactive A1, follow-up B1 testing (either a WB or IFA) is required. If B2 (NAAT) is not performed initially, it may be used to resolve an indeterminate WB or IFA result from persons with possible HIV-1 acute infection. ~~At present, the FDA-approved NAAT is only for use with plasma specimens.~~ All the supplemental tests in Algorithm 2 are approved only for confirmation of HIV-1. Therefore, specimens that are reactive on an HIV-1/2 combination antibody assay, but negative or indeterminate on supplemental testing, could represent potential HIV-2 infections. Algorithm 2 provides more guidance than Laboratory Algorithm 1 to resolve suspect HIV-2 cases. (See Laboratory Algorithm 5 for more information.)

Available Data

Refer to the “Available Data” section of Laboratory Algorithm 1 as that data set is relevant in support of Algorithm 2. The available data supports NAAT as a supplemental test in the confirmatory process and is therefore applicable to both Laboratory Algorithms 1 and 2.

Summary

Benefits

- FDA-approved assays are available for A1, B1 and B2
- Using NAAT (B2) better aligns the sensitivity of screening and confirmatory tests in early infection; and ~~when plasma is available~~ NAAT may confirm HIV infection earlier for repeatedly-reactive A1 tests, eliminating the need to collect a subsequent specimen,
- HIV-1/-2 combination immunoassays reliably detect both HIV-1 and HIV-2 antibodies.
- It provides information to inform when HIV-2 discriminatory testing may be indicated.
- Offering options B1 or B2 for confirmatory testing accommodates laboratories that are not equipped to perform NAAT.

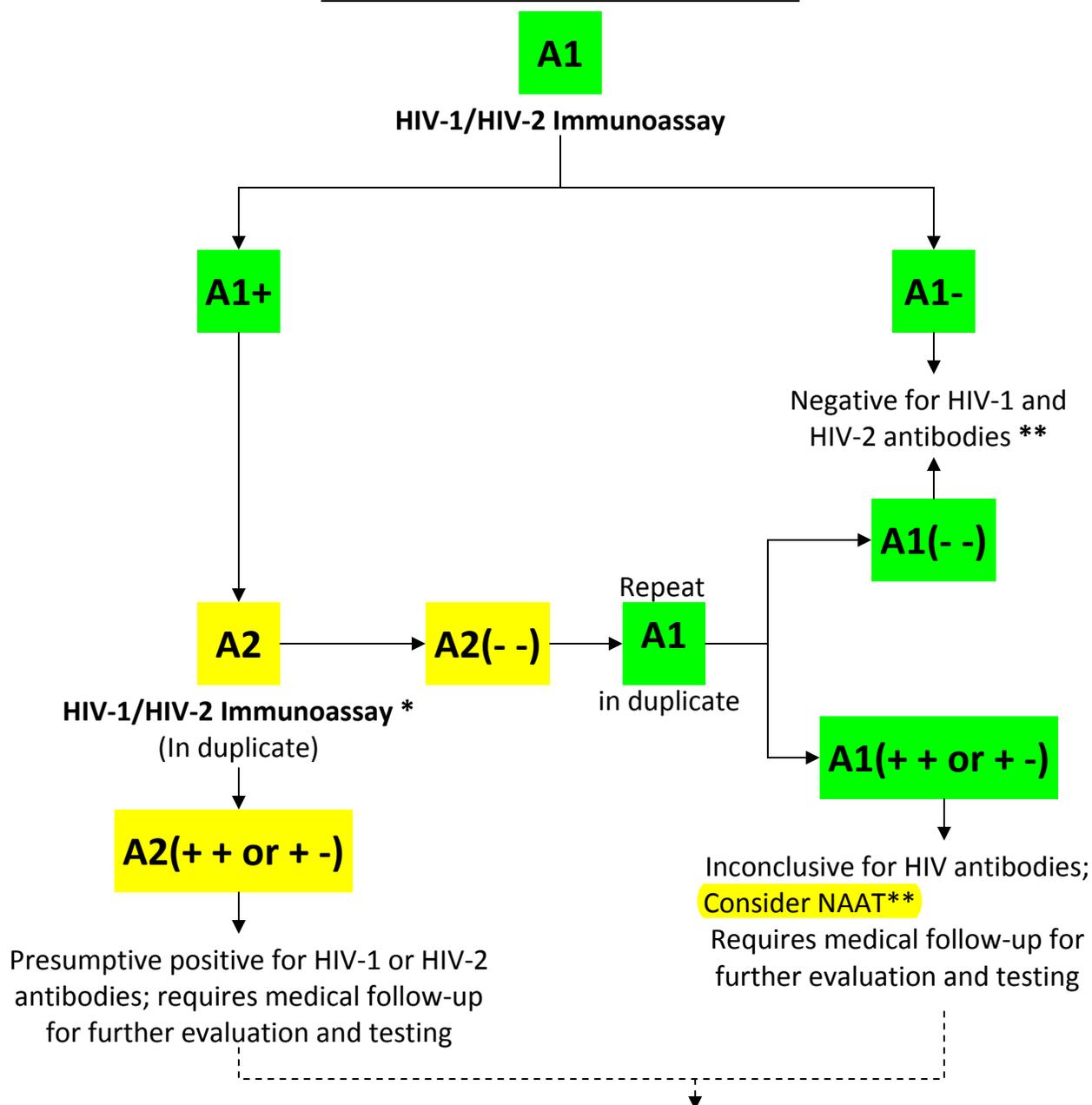
Drawbacks

- A1-RR, B2-negative specimens require further testing with a B1 antibody method.
- ~~Use of B2 (NAAT) requires plasma specimens.~~
- Use of NAAT assays for B2 that are not FDA-approved for diagnosis (e.g., quantitative viral load assays) requires validation by individual laboratories.

Additional Data Needed to Substantiate and Refine the Algorithm

- Number and percentage of initially-reactive A1 immunoassay tests that are not repeatedly-reactive, to determine if it is necessary to repeat a reactive A1 test
- Signal-to-cutoff ratios (S/CO) of initially-reactive immunoassays, and S/CO for repeat testing to validate the utility of this additional information for interpretation
- The performance of NAAT as a confirmatory test after a reactive rapid test
- Additional data to evaluate NAAT as supplemental test. This includes comparison data between tests that detect HIV-1 RNA or DNA (FDA licensed and unlicensed) supplemental antibody tests, or with follow-up for seroconversion.
- Number and percentage of false-negative NAAT results in specimens confirmed positive by a different supplemental HIV-1 NAAT, supplemental antibody test or medical evaluation and follow-up in antiretroviral-naïve patients
- ~~Performance data on qualitative NAAT used with serum instead of plasma to determine whether the specimen types achieve equivalent results.~~
- Number and percentage of false-positive HIV-1 supplemental antibody tests, as determined by follow-up
- Number and percentage of false-negative or indeterminate HIV-1 supplemental antibody tests as determined by NAAT on original specimens and medical evaluation or follow-up for seroconversion
- Logistics of NAAT implementation (specimen type, specimen handling, automation, etc) and the impact on reporting turn-around time and cost

Laboratory Algorithm 3 Dual HIV-1/HIV-2 Immunoassay



* Must be a different EIA, CIA or rapid test

** If a window period infection is suspected based on risk assessment or discordant testing, refer to Acute HIV Infection Testing Laboratory Algorithm 4.

Laboratory Algorithm 3

Dual HIV-1/ HIV-2 Immunoassay

Background

Laboratory Algorithm 3 is the greatest departure from the traditional HIV diagnostic algorithm of a screening immunoassay followed by a supplemental confirmatory test. Thus, this algorithm requires additional data and in-laboratory validation. With several assays available that offer more sensitive testing, Algorithm 3 is designed to incorporate dual immunoassays, maximizing the sensitivity of the algorithm to detect early and long-standing HIV infections while maintaining or improving specificity compared with the traditional method. If both assays have similar sensitivity and specificity, the dual immunoassay Algorithm 3 has the potential to reduce the number of discordant results that would occur with Laboratory Algorithms 1 and 2. The proposed algorithm also is expected to reduce the frequency of indeterminate results. Of foremost concern is whether this algorithm leads to prompt referral to medical care for further evaluation without the requirement for an intervening supplemental test. The recent availability of multi-analyte, random access immunoassays, suitable for processing smaller numbers of specimens without the batching necessary for conventional 96-well plate assays, also makes the dual immunoassay algorithm more attractive.

Key Elements of the Design (*Refer to the Laboratory Algorithm 3 schematic.*)

Algorithm 3 incorporates, in serial use, newer antigen sandwich HIV-1 and HIV-2 diagnostic assays. Antigen sandwich EIAs and chemiluminescent immunoassays (CIA) that both use an antigen bridging format are increasingly becoming the assays of choice in the United States. Both formats can detect IgM and IgG antibodies against HIV. In addition, fourth-generation, combination EIAs detect both HIV antibody and p24 antigen. At present, these new laboratory-based EIAs are used in dual immunoassay algorithms outside the United States, and applications for FDA-approval are expected in the near future.

Other key elements of the dual immunoassay algorithm:

- Algorithm sensitivity is dictated by the initial screening assay, thus the EIA or CIA antibody immunoassay with the best sensitivity should be used as A1 and must be appropriate to handle the user's initial screening volume.
- A negative A1 result is proposed to be reported as negative for HIV-1 and HIV-2 antibodies. If a window period infection is suspected, Algorithm 3 should be followed by Laboratory Algorithm 4 (Acute HIV Infection Testing).
- A2 must be a different EIA or CIA method than A1 with different antigen properties or binding/detection methods to minimize concurrent, non-specific reactivity in uninfected patients. A sensitive rapid test may be appropriate as A2 in some laboratory settings, depending on the laboratory capability, testing volume, multi-platform capacity, costs, etc.
- The recommendation to repeat A1 testing when A2 is repeatedly non-reactive allows re-examination of possible sensitivity/specificity differences between the two assays in the user's setting, and, potentially, to rule out false-positive A1 results near the assay cutoff.
- The suggested interpretation of an A1 and A2 repeatedly-reactive specimen is: "Presumptive positive for HIV-1 and/or HIV-2 antibodies; requires medical follow-up for further evaluation and testing." Data are needed to quantify how many such results occur in uninfected patients before a more definitive interpretation could be reported on the

basis of two concordant serologic tests. However, such “dual reactive” results should ultimately promote referrals to care, because truly infected persons and persons who test false-positive on more than one assay would benefit from clinical follow-up to resolve their HIV infection status.

- The dual immunoassay algorithm does not eliminate inconclusive results (discordance between A1 and A2); however, it should greatly reduce the number of inconclusive results associated with the traditional WB/IFA-based confirmatory algorithm. An inconclusive result requires medical follow-up for further evaluation and testing, such as acute HIV infection testing and perhaps HIV-2 assessment. It may be possible for some laboratories to conduct the additional testing on the initial specimen.
- Algorithm 3 uses assays in non-traditional roles. Notably, three new antigen sandwich immunoassays have received FDA approval for diagnostic use within the last 3-4 years, and data for these assays are limited.

Available Data

At the 2007 HIV Diagnostic Conference, presentations from the Maryland²⁸ and Florida²⁹ public health laboratories and the Department of Defense³⁰ described experiences with the dual immunoassay algorithm. Two of these presentations included data in which at least one of the immunoassays was an indirect EIA. Maryland’s public health laboratory reported data on 4,915 specimens with dually reactive (A1+/A2+) results from an indirect EIA (A1) and an antigen sandwich (A2) immunoassay. Of these, 4,864 were confirmed HIV-1 antibody positive by WB. Subsequent HIV-1 NAAT and HIV-2 testing identified another 33 HIV-positive individuals who were not confirmed by WB. The dual immunoassay algorithm demonstrated a 0.7% increase in sensitivity over Laboratory Algorithm 2 (4864/4897). The positive predictive value (PPV) of the dual immunoassay algorithm was 99.6% (4897/4915) and 27 indeterminate WBs were resolved. In addition, 355 specimens reactive on only one of two EIAs (A1+/A2-) were all NAAT-negative.²⁸

A presentation from the Walter Reed Army Institute of Research reported data on 16,415 A1+ and 4,142 dually-reactive immunoassay (A1+/A2+) specimens on two different indirect EIAs. Of these, 4,009 were confirmed positive by WB for a PPV of 96.8% (4009/4142). The PPVs of the initial EIA vs. dual immunoassays were 24.4% and 96.8% respectively, and using Algorithm 3 would have eliminated the need for 98.9% of unnecessary WBs.³⁰

Florida used two different antigen sandwich immunoassays on 2,765 specimens with 96 dually reactive (A1+/A2+) results, a 2.2% (92/92) increase in sensitivity over the traditional algorithm (90/92). The PPVs of the initial immunoassay vs. dual immunoassays increased from 93.9% to 95.8%.²⁹

The conclusions from the three presentations were similar:

- The serial use of two different immunoassays, especially if they are antigen sandwich assays, had better sensitivity and comparable specificity than using either screening assay followed by WB.
- The evidence showed that performing a secondary immunoassay after a reactive initial immunoassay improved the PPV.

- Combined, these data suggest a dual immunoassay algorithm resolves a significant number, but not all, of false positive results and eliminates the need to perform a significant number of WBs.
- The increase in the algorithm sensitivity identifies early HIV infections, but may not be adequate to detect acute infection unless a NAAT or antigen/antibody immunoassay is included in the algorithm.
- At least one presenter also concluded that this algorithm had the potential to reduce costs and turn-around time for results, depending on assay selection and the use of batching vs. “random access” testing.²⁹

Additional data presented by the American Red Cross at the 2005 HIV Diagnostics Conference provided large numbers of test results on two different antigen sandwich assays conducted on a low prevalence population. In the nearly 17 million tests performed, 7,884 specimens were A1-reactive. All 7,445 (98.4%) specimens that were negative on A2 were also NAAT-negative. Of the 439 A2-reactive specimens, 317 (72%) were NAAT-positive and 122 (28%) were NAAT-negative.²⁴

At the in-person meeting of the Laboratory Algorithm Workgroup, held in June 2007, data were presented from a CDC study showing the ability of the FDA-approved rapid tests to serve as the A2 test, to resolve the A1-reactive results. In this study, of 5,324 total specimens tested, 399 were A1-reactive with an antigen sandwich immunoassay. All of the FDA-approved rapid tests (A2) were negative in all 146 A1-reactive, WB-negative specimens. When repeated on A1, eight specimens remained reactive and would have been referred for additional testing. Of the 253 A1-RR/WB-reactive specimens, 243 were reactive on all A2 rapid tests, but ten were non-reactive by one or more rapid tests. Therefore, of the 399 A1-reactive specimens, 18 (4.5%) that were A2- and A1-RR would have required additional testing; ten (56%) of those would have been WB-positive. All A1+/A2+ specimens (n=243) were WB-positive. Thus, specificity of the A1+/A2+ algorithm was 100% in this sample set. These data suggested that rapid tests are able to confirm the majority of true-positive specimens correctly, despite having lower sensitivity than the laboratory immunoassays.³¹

A similar, laboratory-based, retrospective study was conducted in the New York State Public Health Laboratory during 2006-2007.¹² In this study, Multispot HIV-1/2 was performed on specimens sent for confirmation of A1-reactive rapid screening test results. For 174 of these specimens, infection status was resolved as either infected or uninfected by Western blot or—if the Western blot was indeterminate or negative—by RNA testing. The lab determined that 134 specimens were from HIV-infected clients: and all of these were reactive on Multispot. The 134 specimens included one WB-negative client and eight WB-indeterminate clients, but all nine clients had detectable HIV RNA. The remaining 40 specimens were determined to be from HIV-uninfected clients: 35 had negative Multispot test results and 5/174 (2.9%) had concordantly false-positive results, A1+ (POC rapid)/A2+ (Multispot rapid).

In a recent article by Owen et al., a dual-test algorithm and a three-test algorithm were examined using a number of first, second and third generation immunoassays, rapid tests and NAAT assays on an HIV-1 sensitivity panel of 621 HIV-1 positive specimens and on a specificity panel of 513 HIV-1 negative specimens.²⁶ The investigators presented the serial testing data in which results were positive only if both tests were reactive (A1+/A2+). An alternative was also presented in which results were positive (not inconclusive, as defined in Algorithm 3) if either test was

reactive (A1+/A2-). The average differences in sensitivity and specificity between the two dual-test algorithms were 1.1% and 1.4% respectively for the serologic tests only. The author concluded: 1) that the assays used in a dual test algorithm should differ in format or content such that they are not prone to the same false-positive or false-negative results; 2) for either algorithm, test combinations that include antigen sandwich EIAs yield the highest sensitivity/specificity; 3) the data support alternative algorithms that do not include WB, because they produce less ambiguous results, cost less and can meet unique needs of the test population.

Summary

Benefits

- Most A1+/A2+ persons can be referred to care in a timely manner. False-positive results can be resolved by clinical follow-up without the expense of an intervening supplemental confirmatory test (WB, IFA or NAAT).
- The sensitivity of the algorithm may be greater than that of EIA/WB.
- Algorithm specificity is greater than that of a single immunoassay, and comparable to that of the traditional algorithm using WB in high prevalence populations.
- The combination immunoassay algorithm is less costly than those that incorporate supplemental antibody/NAAT tests and likely to have faster turn-around time.

Drawbacks

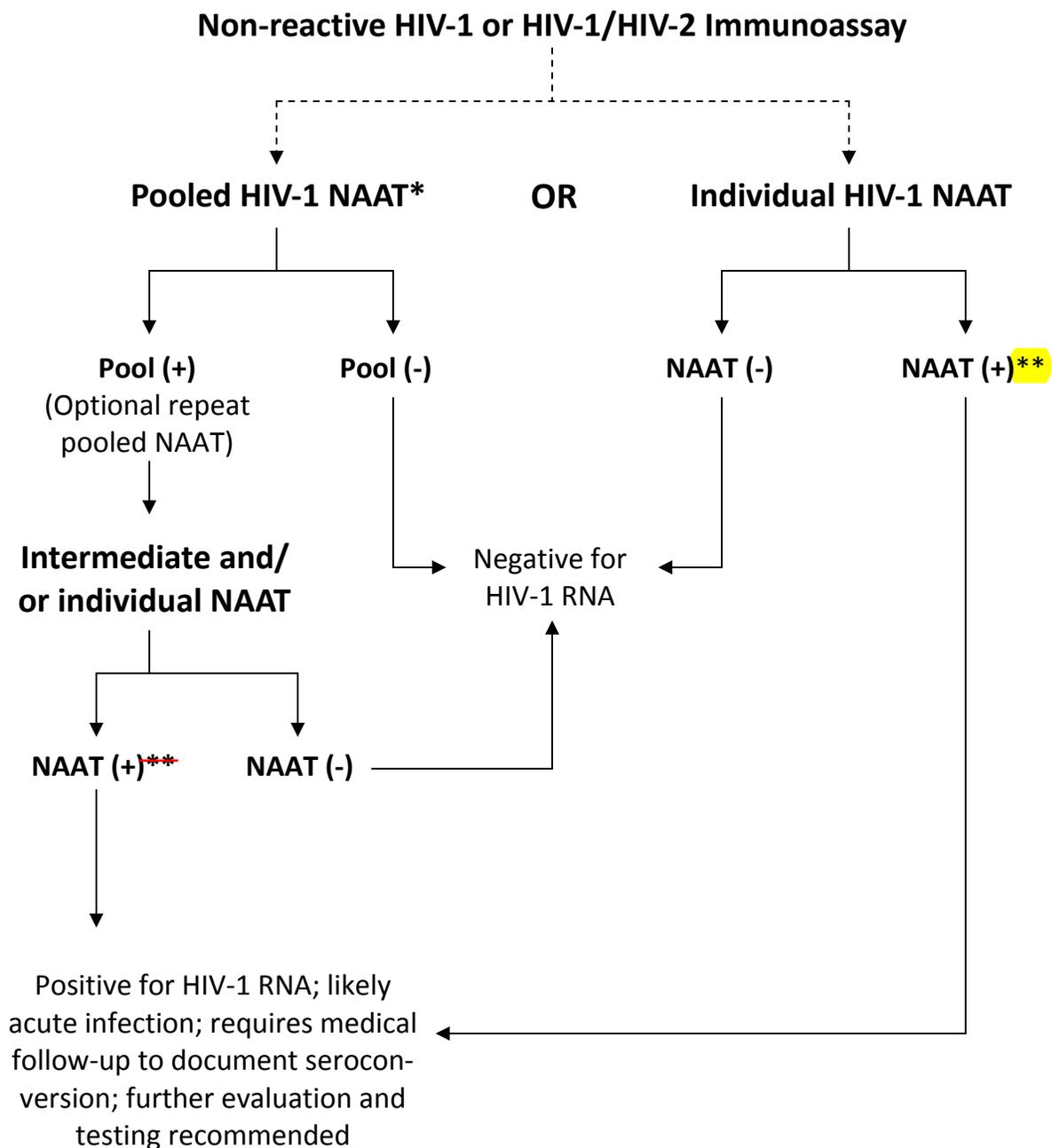
- Concordantly reactive dual immunoassays are interpreted as presumptive positive, and refinements that would allow a more definitive answer are desirable.
- Many current immunoassays require large, expensive automated platforms that may not be practical or feasible for many laboratories.
- Rates of false-positives (A1+/A2+) have not been well-established for specific assays, specific populations, and over a range of HIV prevalence.
- Use of HIV-1/2 immunoassays in a dual immunoassay algorithm will produce lower than expected PPV in low prevalence populations.
- Use of rapid assays for A2 may improve specificity but reduce sensitivity for early infection.

Additional Data Needed to Substantiate and Refine the Algorithm

- Data and/or modifications to the algorithm are needed to advance the “presumptive positive” interpretation of concordant results to a more definitive laboratory-based positive result.
- The need for duplicate testing for either the A1 or A2 test
- Additional data on different rapid tests for A2
- Data for dual combination antigen-antibody detection (fourth generation) assays
- The number and percentage of false-negative A1 results
- S/CO values for both A1 and A2 to validate whether this additional information can be used to improve interpretation of the algorithm
- The number and percentage of A1+/A2-/A1+ results in HIV-infected and uninfected patients
- The number and percentage of A1+/A2+ results in HIV uninfected patients

- Data for specific serial use of specific assays
- Data for the practicality, feasibility and relative cost of the dual immunoassay algorithm in different populations in view of the large, expensive diagnostic platforms required for many of the newer immunoassays
- Performance data for settings with different prevalence and specific populations, such as pregnant women

Laboratory Algorithm 4 Acute HIV Infection Testing



* User validation may be required for pooled testing (Pool size to be determined by user).

~~** It may be necessary to repeat a positive NAAT for confirmation.~~

** Positive NAAT should be repeated for confirmation if antibody is not detectable

Laboratory Algorithm 4 Acute HIV Infection Testing

Background

Studies have shown that the risk of viral transmission to others may be highest immediately after an individual is infected,³² and that persons who learn they are infected with HIV substantially reduce risky behaviors that are likely to transmit the disease.³³ Tests that can detect HIV infection as early as possible after transmission can provide valuable information to patients and potentially help prevent the spread of HIV. NAAT can detect HIV-1 RNA as early as 10-12 days after exposure, whereas the various screening immunoassays detect HIV antibodies 2-6 weeks later.⁴ The US Public Health Service guidelines for antiretroviral therapy recommend screening with an antibody test and individual NAAT in persons with recent high-risk behavior and a compatible clinical syndrome of a viral illness.³⁴

An HIV acute infection algorithm was developed to detect infections earlier, maximizing sensitivity without reducing specificity. Because of the complexity and cost of the NAAT assay, the algorithm is designed as a “reflex algorithm” for use after negative antibody screening in any of the proposed lab-based algorithms. This algorithm can be used for either general or targeted population screening. A major goal for this algorithm is to identify acute HIV-1 infections and facilitate a timely referral to medical care for further evaluation.

Key Elements of the Design (*Refer to the Laboratory Algorithm 4 schematic.*)

The intent of Laboratory Algorithm 4 is to provide guidance for testing for acute HIV-1 infection. Testing pooled specimens allows the screening of large numbers of specimens at reduced costs. Testing of individual specimens with NAAT is useful for patients with symptoms of acute retroviral infection who report recent high-risk exposure. FDA approval of qualitative NAAT for HIV laboratory confirmation of infection, as well as data collected by blood banks and public health laboratories, support the effectiveness of NAAT when used for this purpose. Presenting this algorithm as a “reflex” is due partly to the expense and complexity of NAAT testing. The FDA-approved diagnostic assay is a non-automated, transcription-mediated-amplification (TMA) procedure incorporating chemiluminescent signal detection. The procedure requires ~~a plasma specimen~~, several steps, and can be performed in about 5 hours. The RNA test must be repeated if a reactive RNA result occurs when HIV-1 antibody is not detectable. The following are other elements of the acute HIV infection algorithm to note:

- The focus of the algorithm is to detect HIV-1 acute infections in individuals with negative antibody results from an EIA, CIA or rapid test.
- Individual NAAT is FDA-approved; pooled testing must be validated by the laboratory. Factors such as the number of specimens to reflex test, costs, and validation process may affect path selection.
- Use of the FDA-approved NAAT requires a repeat of individual NAAT-positive results when HIV serology is nonreactive. Repeat testing of a positive NAAT is recommended if a non-FDA approved NAAT is used, ~~but data are needed to validate this recommendation.~~ Repeat testing of an initial-positive pool result is optional, but may be a cost savings measure to rule out false-positive pool results prior to the deconstruction of the pool for individual NAAT testing.

- Data for the size of the master pool and intermediate pools varies. Therefore, the user must validate the entire pooling/testing process.
- A NAAT-positive result should be interpreted as positive for HIV RNA and likely acute HIV-1 infection. Medical follow-up is required to document seroconversion.

Available Data

RNA NAAT has been used for quantifying HIV-1 viral load since the mid-1990s and for qualitative screening of blood donations since 1999. Busch et al., have reported 18 HIV-1 acute infections identified from 50.3 million units of antibody-negative blood screened by a combined pooled NAAT/serology algorithm from 1999-2004.³⁵ However, because NAAT assays are expensive and technically complex, they have not been widely adopted as diagnostic screening tools until recent years.

The North Carolina Division of Public Health adopted an algorithm for pooled NAAT on seronegative specimens obtained from individuals who sought HIV testing through the state's counseling and testing program. To reduce the cost, they choose to use a master pool of 100 specimens with intermediate pools of ten. In the first study of 109,250 individuals who underwent HIV antibody testing, 583 (0.5%) were antibody-positive, and another 23 had acute HIV infections based on antibody-negative serology and RNA-positive NAAT.³⁶ Due to the diagnosis of the HIV-1 acute infections, there was a 4% increase in detected cases of HIV overall.

Findings from other studies performed in San Francisco, Los Angeles and Seattle were similar to North Carolina. They demonstrated an increase of 3.4% - 8.6% in reported HIV cases with the additional diagnoses of HIV-1 acute infections missed by serologic testing.^{37,38} It is important to note that in these studies the EIA used to screen for antibodies was usually the first generation Vironostika assay, with lower sensitivity for detection of early infection.²⁶ Also, both the NAAT assay used and pooling sizes varied widely in these studies. A recent study by Florida, New York State, New York City and Los Angeles examined the performance and benefits of NAAT when the initial screening was performed with a more sensitive antigen sandwich immunoassay.³⁹ The study has concluded recently: unpublished data suggest that incorporating pooled NAAT in an algorithm after an antigen sandwich assay will identify acute infection, but the yield will be less than in the earlier studies.

At the 2007 HIV Diagnostic Conference, presentations from New York, Florida, San Francisco, Los Angeles, New York City, and a commercial laboratory described experiences with algorithms that incorporated NAAT, either for general or targeted screening.³⁹⁻⁴² Different groups reported a wide range of pool sizes (5, 10, 16 and 512). In addition, data from several presentations concluded that a higher yield of acute infections is expected through targeted screening with NAAT, such as in populations at high-risk or in high prevalence areas. Some US sites (San Francisco and New York City) have adopted a targeted, pooled NAAT strategy; therefore, more data will become available in the near future.

Summary

Benefits

- Individual NAAT can identify acute HIV infection in antibody-negative persons with a compatible clinical syndrome, and in persons seeking testing after a recent high-risk exposure.
- Pooled NAAT can identify HIV infection in antibody-negative persons, and pooling reduces the cost of NAAT screening. The yield of NAAT screening depends on the incidence of new infections (often proportional to prevalence in the tested population) and the seroconversion sensitivity of the immunoassay used for antibody screening.
- Pooled NAAT testing of blood donors reduces the risk of transfusion-related HIV infection.

Drawbacks

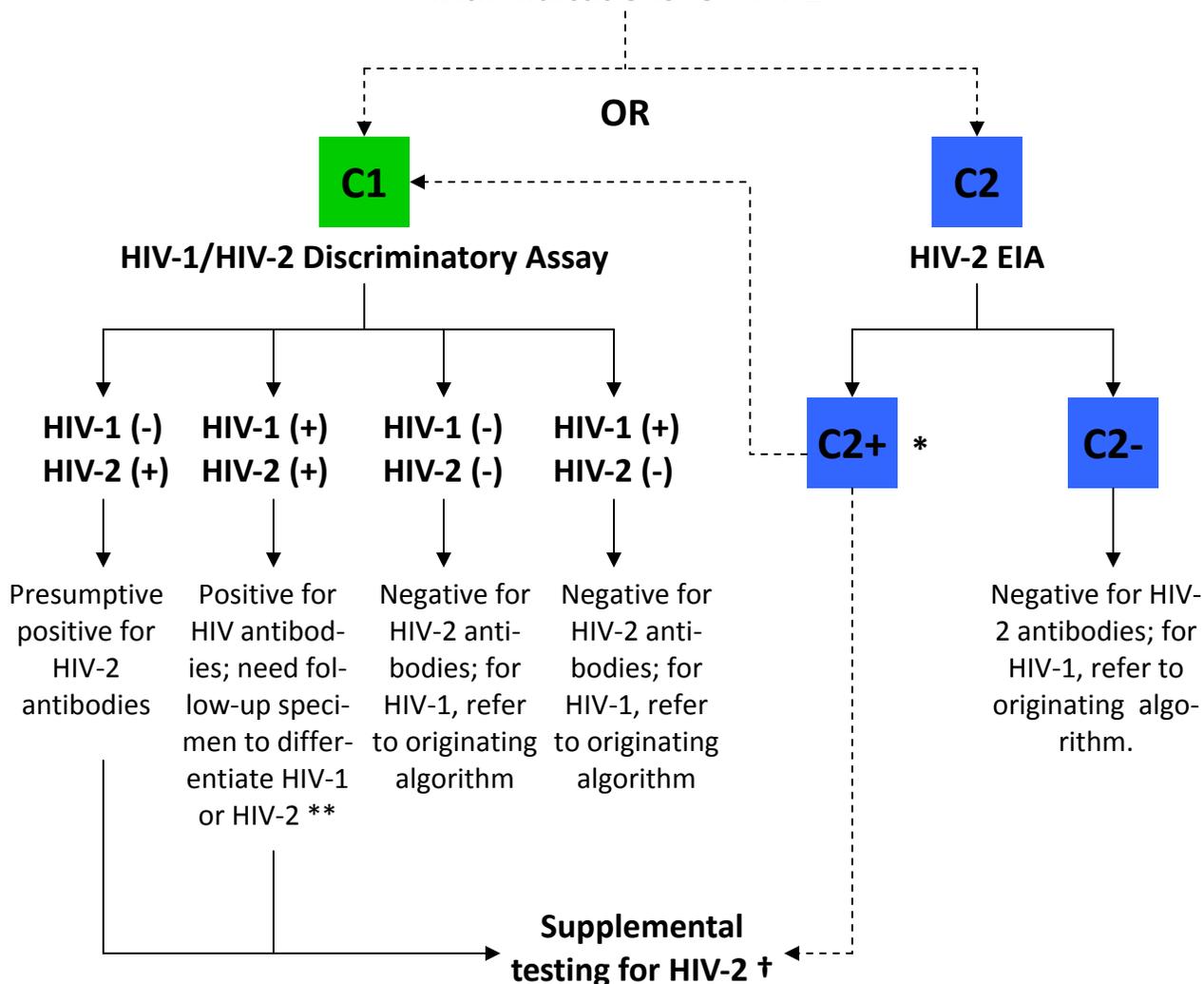
- Pooled NAAT testing increases the cost and turn-around time of HIV screening compared to antibody testing alone. Testing volume, cost and turn-around time for results are the main determinants for selecting pool size.
- The qualitative NAAT test currently FDA-approved for diagnosis of acute HIV infection is not approved for pooled testing. Thus, each laboratory needs to perform a validation with its selected pool size.
- The use of NAAT tests without FDA-approval for diagnostic use must include user validation on pooled testing, as well as individual NAAT testing.
- There may be marginal yield of additional HIV cases compared to 3rd (and 4th) generation immunoassays, particularly in low incidence populations.

Additional Data Needed to Substantiate and Refine the Algorithm

- Analytic and clinical sensitivity of pooled NAAT for various size pools, including threshold pool sizes for detection of HIV-1
- Relative benefits of individual vs. pooled NAAT as the strategy to detect HIV acute infections
- Frequency of false-positive individual NAAT results
- Percentage of individual specimens that are initially reactive on NAAT, but non-reactive on repeat testing of the same specimen with the same NAAT
- Evaluation of the need to repeat NAAT on reactive master pools before deconstruction NAAT testing, and value of signal to cut-off ratios for predicting false-positive results
- Cost-effectiveness of pooled testing with different pool sizes, in populations with different levels of risk and HIV prevalence
- Utility of risk-based criteria to guide NAAT-screening for acute infection
- Validation of optimal pool sizes
- Logistics of NAAT implementation (specimen type, specimen handling, automation, etc) and its impact on yield and reporting turn-around time
- Performance of ~~individual and~~ pooled NAAT on serum vs. plasma specimens

Laboratory Algorithm 5 HIV-2 Testing

Reactive HIV-1/HIV-2 Immunoassay with Indications for HIV-2



* If HIV-2 EIA is reactive, specimen should preferably be tested on an HIV-1/HIV-2 discriminatory or supplemental assay for further clarification. Alternatively, the specimen can be sent to an appropriate public health laboratory with HIV-2 supplemental testing capabilities.

** Dilution testing may be required to differentiate cross-reactivity between strains; refer to package insert of the assay

† Refer to appropriate public health or reference laboratory with HIV-2 supplemental testing capabilities. Because low viral loads are common with HIV-2 infection, testing plasma specimens for HIV-2 RNA is not reliable.

Laboratory Algorithm 5 HIV-2 Testing

Background

The last Public Health Service diagnostic algorithm recommendation for HIV-2 was published in a CDC MMWR on July 17, 1992.² The report provided recommendations for HIV-2 diagnosis in persons tested in non-blood-donor settings and provided guidelines for serologic testing with combination HIV-1/2 screening immunoassays. Data for this report indicated that the prevalence of HIV-2 infections was low, and CDC did not recommend routine testing for HIV-2 in settings other than blood donor screening. However, HIV-2 antibody testing should be considered in cases where a client has: 1) potential epidemiologic risk factors for HIV-2 (sex partners from countries where HIV-2 is endemic; sex partners known to be infected with HIV-2; blood transfusion or non-sterile injection in countries where HIV-2 is endemic; needle sharing with a person from an HIV-2 endemic country or with a person known to be infected with HIV-2; children of women who have risk factors for HIV-2 infection); 2) clinical suspicion of AIDS in the absence of a positive test for antibodies to HIV-1; or 3) an HIV-1 Western blot with unusual indeterminate patterns, such as Gag p55, p24 or p17 plus Pol bands p66, p51 (RT) or p31/32 (integrase). At the time the recommendations were written and still today there is no FDA-approved supplemental test for HIV-2 confirmation.

Key Elements of the Design (*Refer to the Laboratory Algorithm 5 schematic.*)

Laboratory Algorithm 5 is intended for differentiation/confirmation of HIV-2 infections and is designed to be used in conjunction with the results obtained from the other proposed laboratory and point-of-contact algorithms. Currently, there are four criteria that would suggest the need for HIV-2 testing: 1) known local HIV-2 prevalence, 2) client travel or risk history, 3) clinical presentation, such as low CD4 with unconfirmed HIV-1 infection, and 4) indeterminate HIV-1 Western blot results with patterns suggestive of a potential HIV-2 infection, such as Gag p55, p24 or p17 plus Pol bands p66, p51 (RT) or p31/32 (integrase). Due to a lack of FDA-approved diagnostic tests that confirm HIV-2 infection, a definitive algorithm is difficult to establish. However, FDA-approved discriminatory immunoassays and the non-FDA-approved HIV-2 supplemental tests offer great promise for confirmation of HIV-2 infection: they are often used in conjunction with in-house nucleic acid tests at reference laboratories to identify HIV-2 infection.

Available Data

As presented by Kumar et al., at the 2007 HIV Diagnostics Conference,⁴³ CDC maintains a supplemental database for reports of HIV-2 infection. Only 68 confirmed cases were reported between 1996 and 2006. After investigation, the majority of reported HIV-2 infections are still associated with individuals who have ties to West Africa. However, these investigations depend on the reporting of cases to CDC and CDC does not receive reports of HIV-2 infections consistently from all the states. In Maryland, 11 specimens from six individuals have been confirmed as HIV-2 antibody positive between 2004 and 2007.⁴⁴ In New York State, no HIV-2 positive samples have been identified from January 2006 through September 2007.⁴⁵ However, in New York City, 1,388 specimens have been tested for HIV-2 since 1998 and 43 have been confirmed to have HIV-2 infection.⁴⁶ As there are no FDA-approved tests designed specifically

to confirm HIV-2, the studies presented at the conference used various assays for differentiation/confirmation of HIV-2 infection. These included one FDA-licensed test (licensed for discrimination between HIV-1 and HIV-2), a non-FDA approved EIA, and various in-house DNA PCR assays.

The efficacy of HIV-2 detection by FDA-licensed serologic HIV-1 screening tests was reported by George et al., in 1990.⁴⁷ At this time 60-91% of HIV-2 infected persons tested repeatedly-reactive on HIV-1 viral lysate EIAs. A study published in 2008 by Owen et al.²⁶ with a limited number of HIV-2 samples indicated that the current FDA-approved serologic HIV-1 only antibody screening tests detected HIV-2 in 47-100 % of specimens; and screening assays licensed for the detection of HIV-2 detected 100% of the HIV-2 specimens tested. Furthermore, as expected, HIV-1 NAAT did not detect HIV-2 samples. This indicates that a positive HIV-1 NAAT result rules out the need to test directly for HIV-2 infection. While not stated specifically in the manuscript by Owen et al., in this study, all HIV-2 specimens were discriminated as HIV-2 by the FDA-approved discriminatory immunoassay.

Summary

Benefits

- The limited data available indicate that the current FDA-approved and unapproved serologic tests designed to discriminate HIV-1 and HIV-2 infections are effective.
- Non-licensed Western blot and in-house HIV-2 specific DNA PCR assays appear to be effective for discrimination/confirmation of HIV-2 infections.

Drawbacks

- A major hurdle in diagnosing HIV-2 accurately is the lack of an FDA-approved supplemental test. Laboratories are limited to unapproved alternatives that must be validated in-house. Such validations are difficult given the limited patient population size and are required to be compliant with patient test reporting guidelines.
- There are regulatory constraints that need to be overcome: currently approved HIV-2 discriminatory tests are not labeled as confirmatory/supplemental tests.

Additional Data Needed to Substantiate and Refine the Algorithm

There is a need to collect additional known HIV-2 samples to determine the effectiveness of the proposed testing strategy. Better HIV-2 epidemiologic data is also needed to clarify the necessity for HIV-2 testing in US settings.

Specific data needs include:

- Number and percentage of specimens from persons truly infected with HIV-2 (diagnosed by medical evaluation and follow-up or by HIV-2 DNA sequence analysis) that are reactive on an HIV-1/2 antibody screening test, but false-negative on an HIV-1/2 discriminatory assay or HIV-2 only EIA
- Number and percentage of specimens reactive for both HIV-1 and HIV-2 on an HIV-1/2 discriminatory assay that are: truly infected with HIV-1 only; truly infected with HIV-2 only; and true dual HIV-1/2 infections

- Number and percentage of specimens that are truly infected only with HIV-2 that are reactive on an HIV-1/2 antibody screening test and negative on an HIV-1 supplemental test; number of these specimens reactive for HIV-2 on an HIV-1/2 discriminatory assay; number of these specimens reactive for HIV-2 on an HIV-2 only EIA.
- Number and percentage of specimens that are truly infected only with HIV-2 that are reactive on two or more HIV-1/2 antibody screening tests; number of these specimens that are reactive for HIV-2 on an HIV-1/2 discriminatory assay; number of these specimens that are reactive for HIV-2 on an HIV-2 only EIA.

Challenges to Implementing New Algorithms

There are a number of challenges associated with implementing the alternative algorithms, ranging from structural (policy, law) to operational (staff training, validation and quality assurance). Two overriding challenges are the constantly evolving menu of tests as new assays are developed and approved for use, and gaining the acceptance that an assay might be used in a multiple-test algorithm and not specifically as a screening or confirmatory test. Other general challenges to adoption of alternative algorithms include:

- Some states have statutes or regulations that require a Western blot or IFA as a confirmatory antibody test. Thus, adoption of multi-test POC algorithms, the dual immunoassay laboratory algorithm, or one using NAAT in place of WB or IFA will require changes to these statutes or regulations. If all persons with two reactive screening tests are referred successfully to medical care, additional confirmatory testing that meets statutory requirements could be performed in the clinical setting.
- Use of multi-test POC algorithms may be challenging for non-laboratory, non-clinical sites, and will require additional resources for training, monitoring, and quality assurance. Good quality assurance practices are essential for both POC and laboratory algorithms.
- The ability of the algorithms to meet the needs of differing HIV program, medical and laboratory communities should be evaluated.
- Developing appropriate, clear and consistent messages about the meaning of a new algorithm's results and the need for follow-up/supplemental testing and/or medical evaluation is essential.
- In sites where multi-test strategies are used for POC testing, adjustments to systems and processes that link clients with care and prevention may be needed to facilitate referrals to medical care, partner services and other prevention and support services.
- Due to the small number of HIV-2 pedigreed samples available for validation studies, the limited amount of HIV-2 specific testing performed in the United States, and the lack of HIV-2 related epidemiologic data, obtaining specimens for the validation of the HIV-2 Testing Algorithm is a challenge. This requires individual laboratories to perform validation studies in order to be compliant with patient test reporting guidelines.
- Discriminatory assays, whether FDA-approved or not, offer great promise for confirmation of HIV-2 infection and are often used in reference labs with in-house nucleic acid tests for HIV-2 to make a determination of infection; however there is need for further data supporting the use of these tests for HIV-2 confirmation. There are also regulatory constraints, such as package insert labeling, which need to be addressed. The greatest advance in HIV-2 testing would be an FDA-approved, inexpensive confirmatory test, either in the form of a serologic test or an HIV-2 proviral DNA test.

Next Steps

As APHL and CDC solicit feedback from the HIV community on these proposed algorithms, the foremost priority is collecting information that addresses the data needs outlined in this report. Contact hiv.algorithm@aphl.org with questions or comments about the proposed algorithms, or to share information that helps meet the data needs. Subject matter experts will respond. APHL and CDC will not disseminate any data without the express permission of the investigators. The APHL/CDC HIV Steering Committee will coordinate the efforts to address the priorities listed below. Priority activities include:

- Continue to solicit feedback on the proposed algorithms from a broad audience
- Conduct comparisons of POC and laboratory assays to state-of-the-art immunoassays (i.e., sandwich assays)
- Encourage comparisons of current and alternative algorithms. Provide a forum to collect data from the evaluations of the alternative algorithms and from attempts to address the specific data needs still required for each algorithm.
- Determine the value of signal-to-cutoff ratios in assisting with HIV test interpretation
- Determine whether or not an initially-reactive serologic screening test or NAAT needs to be repeated.
- Review Western blot interpretation criteria and the value of Western blot results in the identification of infected individuals. (This review is underway and feedback from clinical and commercial laboratories, as well as public health laboratories and program managers, will be solicited.)
- Evaluate performance of the algorithms in different populations with varying HIV prevalence rates and risk factors.
- Provide guidance in the development of appropriate messages to accompany algorithm results.
- Assess the impact of the use of alternative algorithms on reporting, referral to care, quality assurance efforts, varying regulations, HIV case definition reform, etc. Additionally, special attention should be paid to POC and laboratory communication and collaboration so that appropriate laboratory referrals are made after POC testing.
- Encourage manufacturers to bring new assays, as well as new indications for use of existing assays, to the FDA
- Encourage manufacturers to specify in the package insert HIV antigens used in the assays

- Monitor new tests approved by FDA to assess whether modifications in the proposed alternative algorithms are needed
- Plan to share new data at the next APHL/CDC HIV Diagnostics Conference to be held in Orlando, Florida March 24-26, 2010
- Coordinate efforts with the Clinical and Laboratory Standards Institute (CLSI) initiative to develop, “Criteria for Laboratory Testing and Diagnosis of HIV-1 Infections.”

CDC and APHL elected to publish this report on the status of the proposed algorithms jointly in an effort to reach a broader audience and to solicit feedback from a range of HIV professionals. In order to update the menu of HIV testing algorithms, your feedback and data are essential. These improved algorithms will lead to the ultimate goal of increasing the number of individuals tested accurately and, if infected, referred to care as soon as possible.

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