

CONFERENCE CALLS FOCUS ON NEW TRENDS IN HIV DIAGNOSTICS

The Role of PHLs

In February 2011, the Association of Public Health Laboratories (APHL) hosted a series of member conference calls to discuss current issues and trends in HIV diagnostics. Each conference call was attended by five to ten laboratories and facilitated by members of the APHL/CDC HIV Steering Committee. Participants were able to ask questions about new diagnostic technologies, share stories from their laboratories, and discuss the barriers and benefits to implementing the new HIV testing technologies and proposed testing algorithms.

The landscape of HIV testing in public health laboratories (PHLs) has changed over the past two years. Rapid testing at the point of care has continued to grow, an HIV-1 EIA for oral fluid and dried blood spot specimens received FDA approval, and the first fourth generation HIV-1/2 antigen/antibody immunoassay (IA) entered the US market. (This type of assay is capable of identifying infection prior to seroconversion by detecting the viral p24 antigen, and could further reduce the window period of detection.)

In anticipation of the arrival of antigen/antibody immunoassays, and in response to the limitations of the current HIV testing algorithm, which has been in place since 1989, a new HIV diagnostic algorithm was proposed at the 2010 HIV Diagnostics Conference. The current algorithm calls for a specimen repeatedly

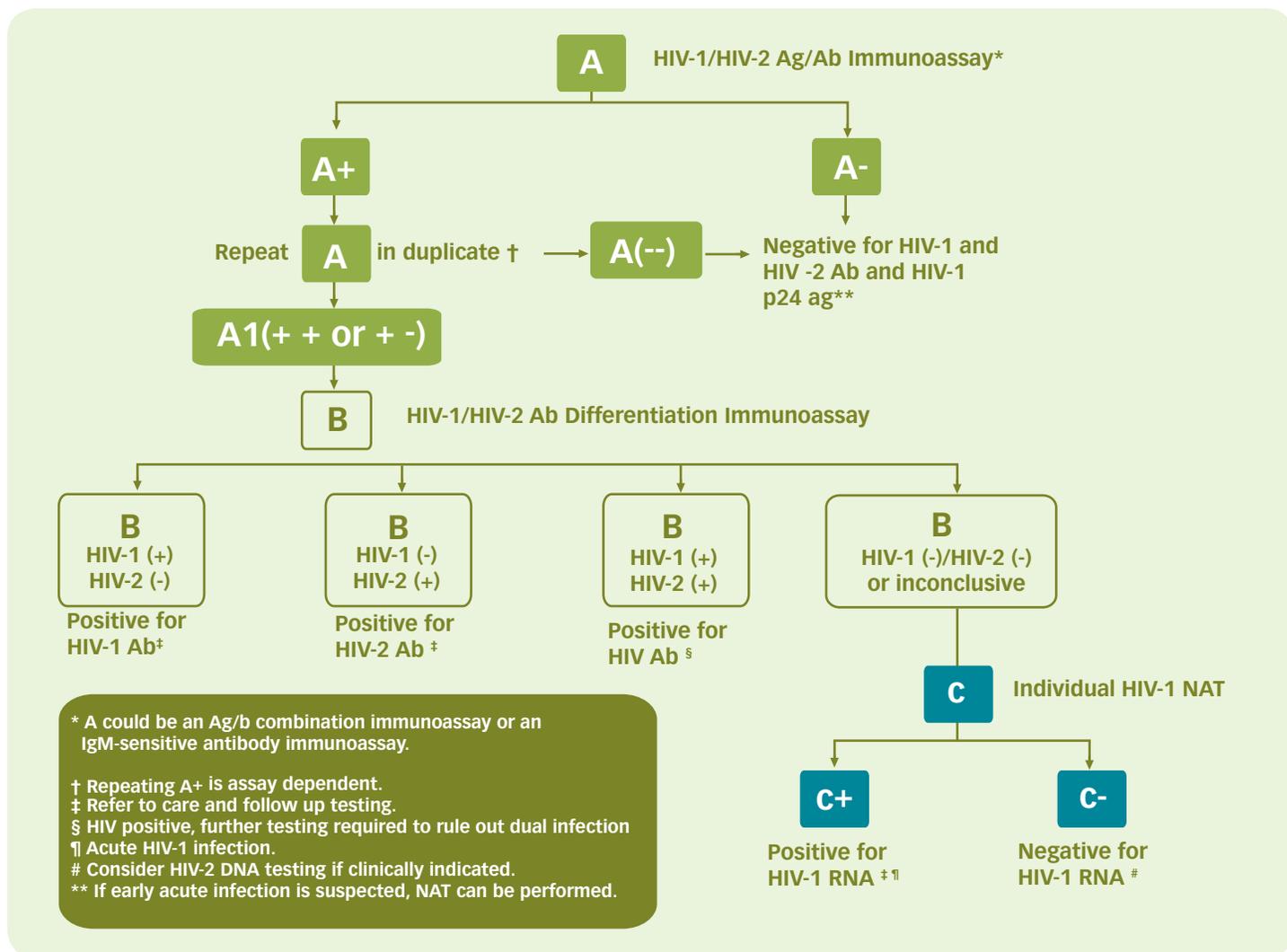
reactive by immunoassay to be confirmed by a supplemental HIV antibody test—that is, Western blot (WB) or indirect immunofluorescence assay (IFA). As testing technology has improved to detect earlier infections, the supplemental tests have remained the same, resulting in more specimens that require follow-up and additional testing. The new algorithm, shown in Figure 1 (page 2) makes use of more advanced testing technologies including a fourth generation HIV-1/2 Ag/Ab immunoassay, an HIV-1/HIV-2 antibody differentiation assay, and nucleic acid amplification tests (NAAT), with the goal of identifying more infections earlier and differentiating HIV-1 from HIV-2. This algorithm also has the potential to decrease costs, improve turnaround time, and result in fewer indeterminate results. APHL, CDC and several partners are currently collecting data to evaluate the performance

of this algorithm. Some of the pros and cons of this proposed strategy are summarized in Figure 2 (see page 4).

APHL convened a series of conference calls for public health laboratorians to introduce the new algorithm, discuss benefits and barriers to implementation in PHLs, and talk about the future direction of HIV testing in PHLs. From February 1 through February 24, APHL hosted seven conference calls with APHL members. Altogether, 64 member laboratories participated in these calls, including 42 state and 22 local PHLs.

Each conference call was moderated by HIV Steering Committee members and began with a brief slide presentation on recent developments in HIV testing technology and introduced the proposed diagnostic laboratory algorithm. Following the presentation, Steering Committee members moderated an open Q&A session in which participants could ask questions about the proposed algorithm, share stories from their laboratories, and discuss some of the issues they currently face. This issue brief summarizes the major topics discussed during these conversations.

FIGURE 1: PROPOSED LABORATORY ALGORITHM



NOTE: Algorithm proposed at the 2010 HIV Diagnostics Conference, March 2010.

TRENDS IN TESTING VOLUME

Several of the conference call discussions centered on HIV testing volume in PHLs. Many of the participating PHLs indicated that they have seen a significant decrease in the number of specimens (serum/plasma) submitted to their laboratories for HIV testing. These observations correlate with data collected from the 2006 and 2009 APHL laboratory surveys, which indicated that participating PHLs (state and local) saw a 22.2% decrease in total HIV testing volumes over that time period.

Since the arrival of CLIA-waived rapid tests, HIV screening at the point of care has steadily increased.

TRENDS IN ORAL FLUID TESTING

The subject of HIV-1 oral fluid screening and confirmation was not a planned topic of discussion on the conference calls; however, the issue was often raised by participants, and a few surprising trends were noted.

At least one third of the call participants performed HIV-1 oral fluid testing in some capacity. Approximately half of these indicated their oral fluid testing volume had increased in recent years, ranging from 25% - 80%. Much of this increase was attributed to public health programs' preference for collecting oral fluid over blood specimens at many testing sites.

Despite the fact that a first generation immunoassay (IA) for oral fluid specimens was FDA approved last year, several laboratories stated they would continue to use a third generation blood-based IA off-label for screening oral fluid specimens. The primary reason for their decision was that they did not want to maintain two separate screening platforms: one for serum/plasma and another for oral fluid. They also noted that the FDA-approved test for oral fluid is a first generation, HIV-1 viral lysate immunoassay that is less sensitive on serum/plasma specimens during early infection and is not automated. However, some laboratories, especially those that test a significant volume of oral fluid specimens, have converted or plan to convert to the FDA-approved assay for oral fluid screening. Factors influencing this decision included state regulations about reporting results from off-label tests and the efforts involved in validating an assay for off-label use according to CLIA requirements.

Several participants on the conference calls indicated that the decrease in testing volume is a barrier to the implementation of the proposed testing algorithm in their laboratories. An interesting comment made during the discussion was the observation that perhaps HIV screening will eventually move out of the PHLs, where the focus may need to be on supplemental testing, testing for disease management or other advanced techniques.

The remaining call participants indicated their oral fluid testing volume had either leveled off or decreased, primarily because their overall HIV testing volume had decreased. The overall decrease in testing has been attributed to expanded testing at the point of care. Many of these laboratories indicated they would consider switching to the FDA-approved assay, as its manual operation would be suitable for their smaller test volumes.

A couple of laboratories have discontinued oral fluid screening completely, and are now only conducting the oral fluid Western blot in house. Others are requesting that testing sites conduct a blood draw following a reactive oral fluid rapid test.

Many participants indicated that they would like to see HIV-1/2 Ag/Ab immunoassay manufacturers pursue FDA approval of their assays for use with oral fluid specimens. Although oral fluid specimens would still require a separate diagnostic algorithm (because the supplemental tests, B and C, require blood), a screening IA suitable for multiple specimen matrices would reduce the number of assays a laboratory would have to maintain. However, at the 2010 HIV Diagnostics Conference, several manufacturers stated that they would not be pursuing FDA approval for oral fluid on HIV-1/2 Ag/Ab assays because they see oral fluid as an inferior specimen type.

FIGURE 2: QUESTIONS ABOUT THE PROPOSED ALGORITHM

PROS	CONS
<ul style="list-style-type: none"> ■ Detects acute, as well as established, HIV infections ■ Differentiates HIV-1 from HIV-2 ■ Get timely results to facilitate initiation of care/more same-day reporting ■ Eliminate indeterminate and inconclusive results whenever possible ■ Decreased tech time, potential cost savings 	<ul style="list-style-type: none"> ■ Need a separate algorithm for Oral Fluid and Dried Blood Spots ■ Current cost of 4th generation screening platforms ■ Low volume of specimens will require NAAT, creating the challenge of maintaining a low volume assay or sending HIV NAAT to a reference lab ■ Few options currently available for tests in the proposed algorithm

NOTE: Pros and cons of the proposed algorithm (Fig. 1) compared to the current strategy.

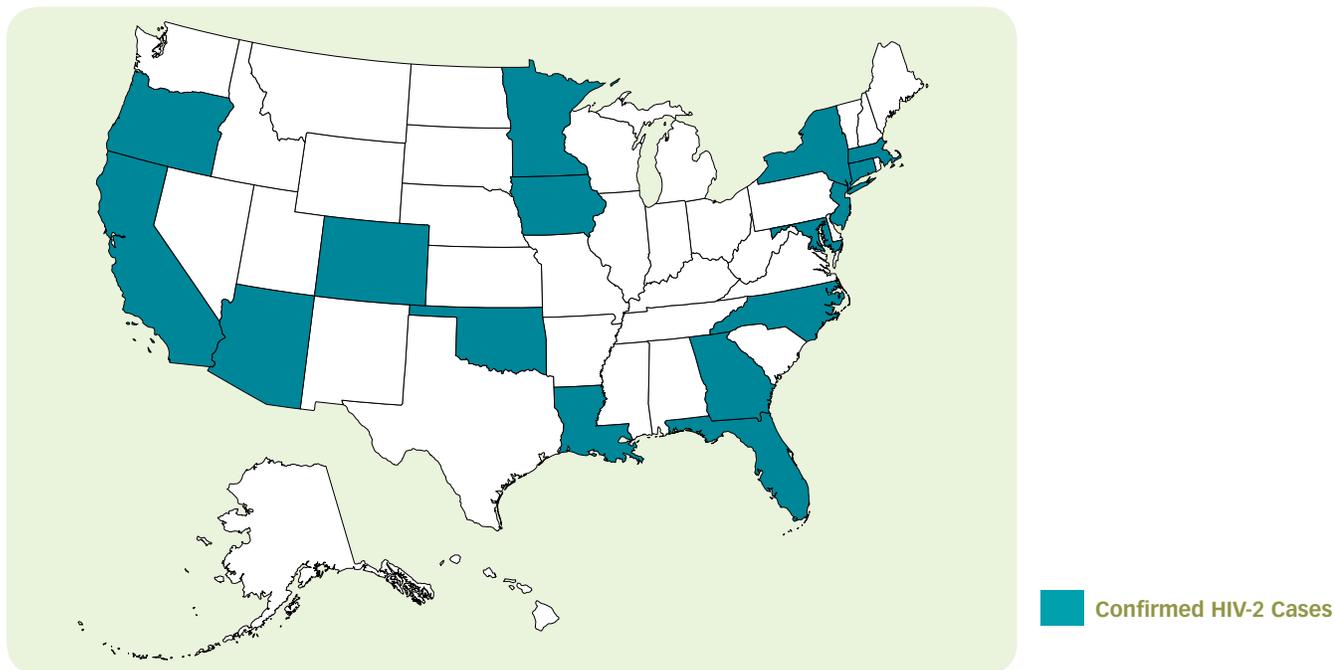
Is there a need for HIV-2 testing capacity?

Although HIV-1 infection is predominant in the US, HIV-2 continues to be reported. HIV-2 cases have been confirmed in at least 17 states in the past 3 years, including low prevalence areas of the US (see Figure 3). Although most cases of HIV-2 have been reported from New York, where specific testing for HIV-2 is performed routinely, cases of HIV-2 have been reported from all regions of the US.¹ HIV-2 testing should be considered based on relevant epidemiologic and laboratory findings,² but HIV-2 should also be considered whenever an HIV-1/HIV-2 initial assay is reactive. HIV-2 may be misdiagnosed as HIV-1 due to extensive cross reactivity on Western blot.³ Between 2000 and 2008 in New York City, 64.5% (40/62) of those diagnosed with HIV-2 were initially reported as positive for HIV-1; furthermore, 85% of HIV-1 Western blots conducted on patients with HIV-2 were positive.⁴ Accurate diagnosis and HIV-1/2 differentiation is important to clinical care and treatment, since many commonly used antiretroviral drugs (e.g., NNRTIs and fusion inhibitors) are ineffective against HIV-2.⁵

Is it important to test for acute HIV-1 infection?

In high incidence settings, testing for acute infection has increased the number of detected cases by 3-10%. However, few data are available for most settings.^{6,7,8,9,10} Data have been published demonstrating the relative transmission probabilities of HIV during the recent or acute phase, compared to long-standing infection. These data show that infectiousness is very high during the acute phase.^{8,11} From a public health point of view, the ability to detect such infections should be considered to be of great importance. Antigen-antibody tests can detect ≥80% of acute infections that are negative for antibody, and will most likely have comparable prices to third generation (antibody-only) tests. Moreover, such tests will exist in both manual and automated platforms. For these reasons, the extra capability of antigen/antibody tests might be available at little additional cost to the laboratory.

FIGURE 3: HIV-2 CASES CONFIRMED AT CDC, 2008-2010



NOTE: Data courtesy of Dr. Michele Owen, from CDC's HIV Diagnostic Laboratory, 2008-2010

Will a 4th generation antigen-antibody assay be required as the screening test?

The proposed laboratory algorithm recommends an HIV-1/2 Ag/Ab immunoassay as the initial screening test in order to maximize the algorithm sensitivity; however, because of limited assay availability and potential cost issues, one may consider using an IgM-sensitive third generation immunoassay as the primary screening test. This option was a popular discussion topic during the calls.

Participants saw the need to screen for both acute and long-standing HIV-1/2 infections, but most laboratories were concerned about the cost differential between third generation and fourth generation tests. Only a few laboratories were actively validating and/or converting to the one currently approved HIV-1/2 Ag/Ab immunoassay for initial screening. These laboratories were either involved in a research project requiring the assay or they already had the required operating platform in-house, limiting the conversion cost. A few laboratories indicated that they preferred the use of a “random access” screening platform because it allows for a faster reporting turnaround time.

Since many specimens received in PHLs are already pre-screened reactive by rapid test, the benefit of adding a HIV-1/2 Ag/Ab screening assay may be diminished. While many HIV program coordinators and clients prefer the convenience of rapid testing, some high-incidence sites could benefit from implementing laboratory-based acute screening. Currently, some programs screen for acute infection on specimens that test negative for antibody with rapid tests. To convince test requesters or program managers of the benefit of screening for acute infection, planning and outreach will be needed.

Will the proposed algorithm meet the surveillance case definition?

The current case definition for HIV dates back to 1993 and was last revised in 2008. It calls for a positive result from an HIV antibody screening test (e.g., reactive enzyme immunoassay) to be confirmed by a positive result from a supplemental HIV antibody test (e.g., WB or IFA) or from an RNA or DNA test. The case definition is intended for surveillance and not patient diagnosis. To accommodate the proposed algorithm, the surveillance case definition will require revision. CDC is currently working towards making the changes for HIV-1 and HIV-2 surveillance so that the proposed algorithm would satisfy the case definition.

The proposed algorithm recommends nucleic acid testing for specimens that are not resolved by the first two tests. Can a quantitative nucleic acid (viral load) assay be used in this capacity?

In the proposed algorithm, NAAT is used if an initially reactive specimen is not reactive on the second test (the HIV-1/HIV-2 discriminatory assay). The discussion on the conference calls primarily focused on which type of NAAT assay was more appropriate for use as the third test in the proposed algorithm: qualitative or quantitative. There was an overwhelming concern as to the increased costs associated with including NAAT, even when the estimated number of specimens requiring such testing may be low in most populations. The consensus among the participants on the latter concern was to consider partnerships, shared-service models or reference laboratories for HIV NAAT, regardless of the methodology used.

When questioned about HIV-1 viral load testing in their laboratories, at least three participants indicated they performed this testing routinely as a therapeutic management service, and three others as a validated diagnostic tool with individuals at high risk of acquiring HIV-1 infections. Several barriers to using an HIV-1 viral load assay as the third test were raised during the conference calls:

1. The process of validating a quantitative assay for diagnostic use would be too involved and should be the responsibility of the manufacturer.

2. Because results of different viral load assays do not often correlate with one another, perhaps a quantitative threshold value for diagnosis could be validated (i.e., a value below the threshold would be considered indeterminate).
3. Since most viral load assays require plasma, the specimen of choice for the algorithm would be plasma; this could be a logistical issue in converting from long-standing serum use.
4. Sample stability studies should be included in validating viral load assays for diagnostic purposes because diagnostic samples are not typically handled within the same timeframe as plasma specimens submitted for therapeutic monitoring purposes.
5. There were concerns about lack of laboratory space for new NAAT platforms.

When can we expect guidance from APHL and/or CDC?

Several participants on the conference calls indicated that official guidance would be necessary prior to switching to a new testing algorithm. APHL and CDC are in the process of gathering data to evaluate the performance of the proposed algorithm. Guidelines for use of supplemental tests after HIV-1/2 Ag/Ab immunoassays are anticipated within the next year, as well as updated recommendations for diagnosis of HIV-2.

BARRIERS AND BENEFITS TO IMPLEMENTING A NEW DIAGNOSTIC ALGORITHM

While the proposed algorithm offers several improvements over the current HIV diagnostic algorithm, there are also some concerns (as outlined in Figure 2). Chief among these concerns is cost. CDC is currently conducting an analysis to determine whether the proposed algorithm might offer cost savings, despite the higher price of HIV-1/2 Ag/Ab assays compared to antibody-only tests. While the Abbott HIV-1/2 Ag/Ab immunoassay system has been available as an FDA-approved method for HIV screening since June 2010, an immunoassay from Bio-Rad has also been submitted for FDA approval. Data regarding the Abbott product's ability to detect acute infection is published.^{12,13} When the Bio-Rad product gains FDA approval, a cost comparison could take place.

*On July 25th, Bio-Rad announced the approval of their 4th generation GS HIV Combo Ag/Ab EIA.

As mentioned previously, NAAT capability was also considered a challenge in many laboratories. The majority have not yet implemented the qualitative NAAT assay currently approved for diagnostic use as a supplemental test. As the number of specimens requiring NAAT in the proposed algorithm is expected to be low, bringing on this assay may be challenging. Validating a different NAAT, such as a quantitative viral load assay, could be a solution for some laboratories. Others have discussed partnering with clinical laboratories in their jurisdictions or establishing shared-services agreements with neighboring PHLs.

While offering NAAT may present a challenge, the ability to confirm acute infections, efficiently link patients to care, and reduce the occurrence of indeterminate supplemental results offer significant

enhancements for both the laboratory and for public health outcomes.

The 2011 Public Health Laboratory HIV Conference Calls provided an excellent forum for discussion of the future role of PHLs in HIV testing. As APHL and CDC work toward updating the recommendations for HIV testing, the input and support from APHL members will continue to be a vital resource.

CDC REFERENCE LABORATORY

Reference laboratory resources are available at the CDC for public health laboratories seeking assistance in evaluating suspect HIV-2 reactive specimens and other difficult or unusual specimens. Contacts for questions regarding the types of specimens and the submission information needed are Michele Owen, mowen@cdc.gov or 404.639.1046, and Tim Granade, tgranade@cdc.gov or 404.639.3850.

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