

HIV DIAGNOSTICS SURVEY

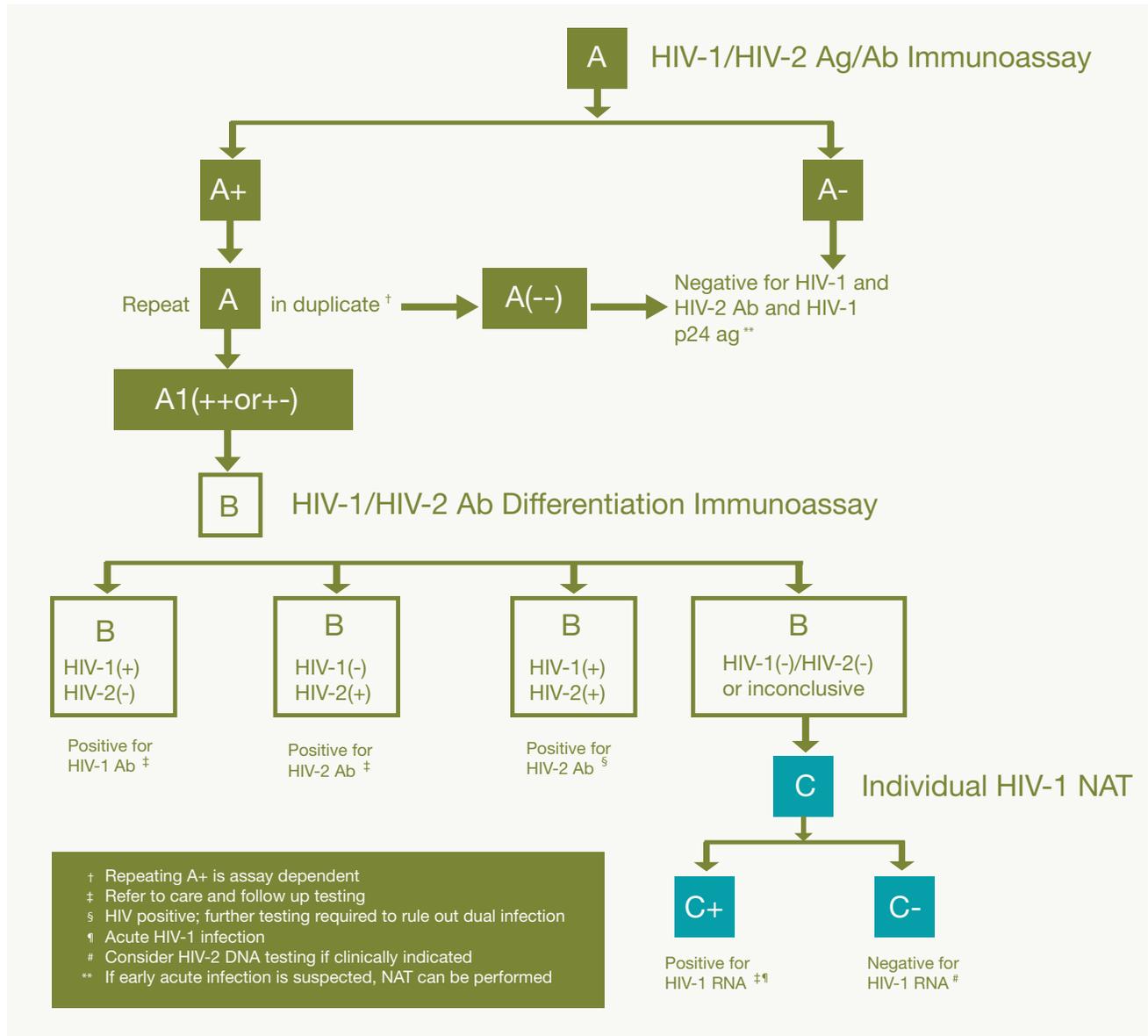
The Association of Public Health Laboratories (APHL) periodically surveys its members to assess their HIV diagnostic testing capabilities, capacities and practices.^{1,2} Past surveys have documented changes in the types of specimens laboratories receive, the test kits they use, and the testing strategies that they implement. Since 2009, when the last survey was conducted, the Centers for Disease Control and Prevention (CDC) and APHL proposed a new HIV diagnostic testing algorithm, and the US Food and Drug Administration (FDA) approved two fourth-generation HIV immunoassays. Both events have generated the beginning of significant changes in the way HIV testing is conducted.

In March 2010, at the HIV Diagnostics Conference, CDC and APHL proposed a new HIV diagnostic testing algorithm³ (see Figure 1). The new algorithm recommends initial testing with a fourth-generation HIV-1/2 immunoassay (IA) which, if reactive, is followed by supplemental testing with an HIV-1/HIV-2 antibody differentiation assay. Specimens negative or indeterminate by the HIV-1/HIV-2 differentiation assay undergo a nucleic acid test (NAT). This marks the first time that Western blot (WB) was not included in the HIV testing algorithm, since its introduction in 1989. Subsequently, the proposed algorithm was described in the Clinical and Laboratory Standards Institute's (CLSI) *Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline (M53-A)*, which was published in June 2011. Selected validation data for using the algorithm after an initial third or fourth generation assay were published in a December 2011 supplement in the *Journal of Clinical Virology* titled "Update on HIV Diagnostic Testing Algorithms."

Another major development that occurred since 2009 was the FDA approval of the first HIV antigen-antibody combination immunoassays, referred to in this document as fourth-generation immunoassays. In June 2010, FDA approved the Abbott Architect HIV Ag/Ab Combo assay. The Bio-Rad GS HIV Combo Ag/Ab assay was approved by FDA in July 2011. HIV antigen-antibody combination assays allow for the detection of both HIV p24 antigen and human antibodies to the virus, opening up the potential to identify acute and established HIV infections using IAs. The acute phase of HIV is defined as the period after infection with HIV and before the development of detectable antibodies to the virus, often referred to as the window period.⁴ This phase is associated with a higher likelihood of transmission.⁵

In order to determine how public health laboratories are responding to these changes, APHL launched the 2012 HIV Testing Practices Survey in March 2012. This issue brief summarizes the results of the survey.

Figure 1. Proposed HIV Diagnostic Testing Algorithm



METHODS

In March 2012, APHL fielded its fourth survey to assess the HIV diagnostics capabilities, capacities and practices among public health laboratories in the United States. The survey closed in May 2012. This 20-question online tool was created and administered through Qualtrics, a web-based survey instrument. Unless otherwise specified, “respondents” refers to all survey respondents that conduct HIV testing in their laboratory. Survey questions were grouped into the following categories:

- HIV Specimen Types and Testing Volumes
- HIV Serum/Plasma Testing Strategies
- Trends in Oral Fluid Testing
- HIV Nucleic Acid Testing Practices
- Adoption of New Testing Methods
- Impediments to Improved HIV Testing

Respondents were asked to provide data on current (2012) HIV testing practices and HIV testing volumes during the 2011 calendar year (i.e., January 1, 2011 through December 31, 2011); therefore, all responses are for this time period, unless otherwise indicated. The Association launched the survey to 130 state, territorial and local public health laboratories that included, for the first time, laboratories that were not members of APHL.

HIV SPECIMEN TYPES AND TESTING VOLUMES

In 2011, the total number of specimens received for HIV testing by the 65 responding public health laboratories was 1,569,515. The average number of specimens received per laboratory was 24,146. The lowest number of specimens received by responding public health laboratories was 64, and the highest was 235,919. The majority (71%) of specimens received was serum (53%) or serum/plasma (unable to distinguish) (18%). Whole blood accounted for 20% of the specimens received, while only 3% of the specimens received were plasma, and 5% were oral fluid.

These data demonstrate the continued trend of decreased HIV testing by public health laboratories (see Figure 2). Based on the subset of 42 labo-

In all, 65 (50%) laboratories responded: 44 of 51 (86%) state public health laboratories and 21 of 79 (27%) territorial and local public health laboratories. The total response rate for all APHL member laboratories was 61 of 88 (69%). The total number of local laboratory respondents included 17 of 37 (46%) APHL local laboratory members and 4 of 42 (10%) non-member laboratories. All 65 respondents indicated that their laboratory conducted HIV testing at the time of the survey.

Data from this survey were compared to previous HIV surveys conducted by APHL in 2004, 2006 and 2009. The 2004 HIV Diagnostics Testing Utilization Survey provided a snapshot of testing practices in July 2004, the 2006 HIV Diagnostics Survey provided testing data for 2005, and the 2009 HIV Testing Practices Survey covered testing from July 1, 2008 through June 30, 2009. In order to properly evaluate trends in testing volume, data were compiled for a subset of 42 public health laboratories that completed the 2006, 2009 and 2011 HIV surveys (n = 9 local and 33 state public health laboratories). All testing practice trends (e.g. test kit usage) were analyzed using all survey respondents unless otherwise noted.

ratories that responded to the three most recent surveys, 1,723,304 HIV specimens were tested for HIV in 2005. This number dropped to 1,338,007 specimens based on the 2009 survey. In 2011, only 1,196,145 specimens were received for HIV testing in public health laboratories. Comparing data collected in previous surveys demonstrates the continued decrease of oral fluid specimens tested in public health laboratories (see Figure 3), although the number of laboratories performing oral fluid testing has varied slightly. Of the 42 laboratories responding to all three surveys, 22 accepted oral fluid specimens in 2006, 25 in 2009, and 23 in 2011. Overall, there was a 76% drop in oral fluid specimens and 31% drop in blood specimens tested in public health laboratories since 2005.

TEST RESULTS REPORTED

Respondents indicated that 28,348 HIV infections were identified, reflecting an overall positivity rate of 1.9%. Also of note is that 10 public health laboratories were reporting acute HIV infections in 2011. Those 10 laboratories identified a total of 248 acute cases of HIV.

The positivity rate in 2011 was slightly higher than the positivity rate for the total number of labora-

tories that responded in 2009 (1.7%) and 2005 (1.4%). Unfortunately, it is difficult to discern what this higher rate signifies as many unknown factors or confounders could be involved in this calculation, including variations in the specific number of public health laboratories responding and variation in the percentage of specimens received following reactive rapid tests.

Figure 2. Comparison of total and oral fluid specimens submitted for testing for 42 laboratories that completed the 2006, 2009 and 2011 APHL HIV surveys

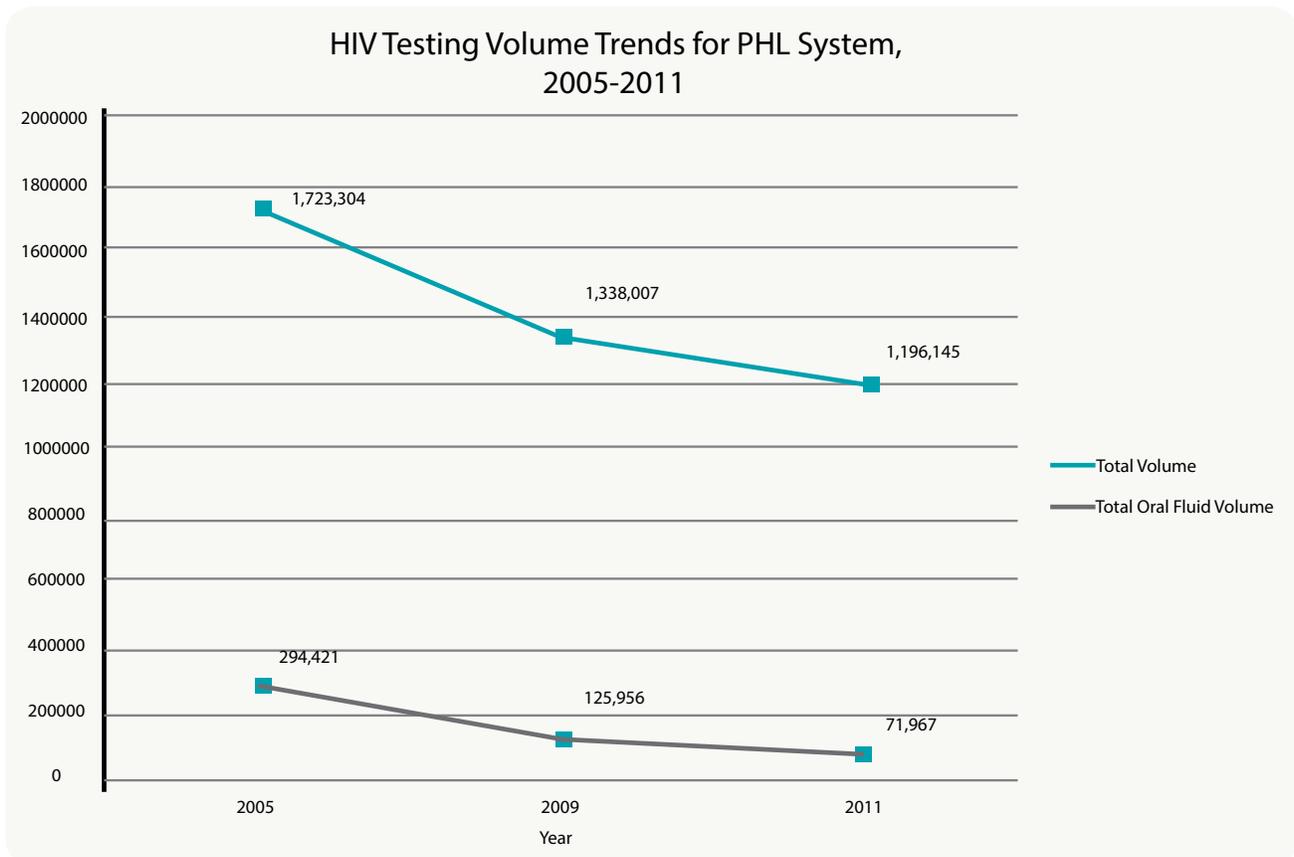
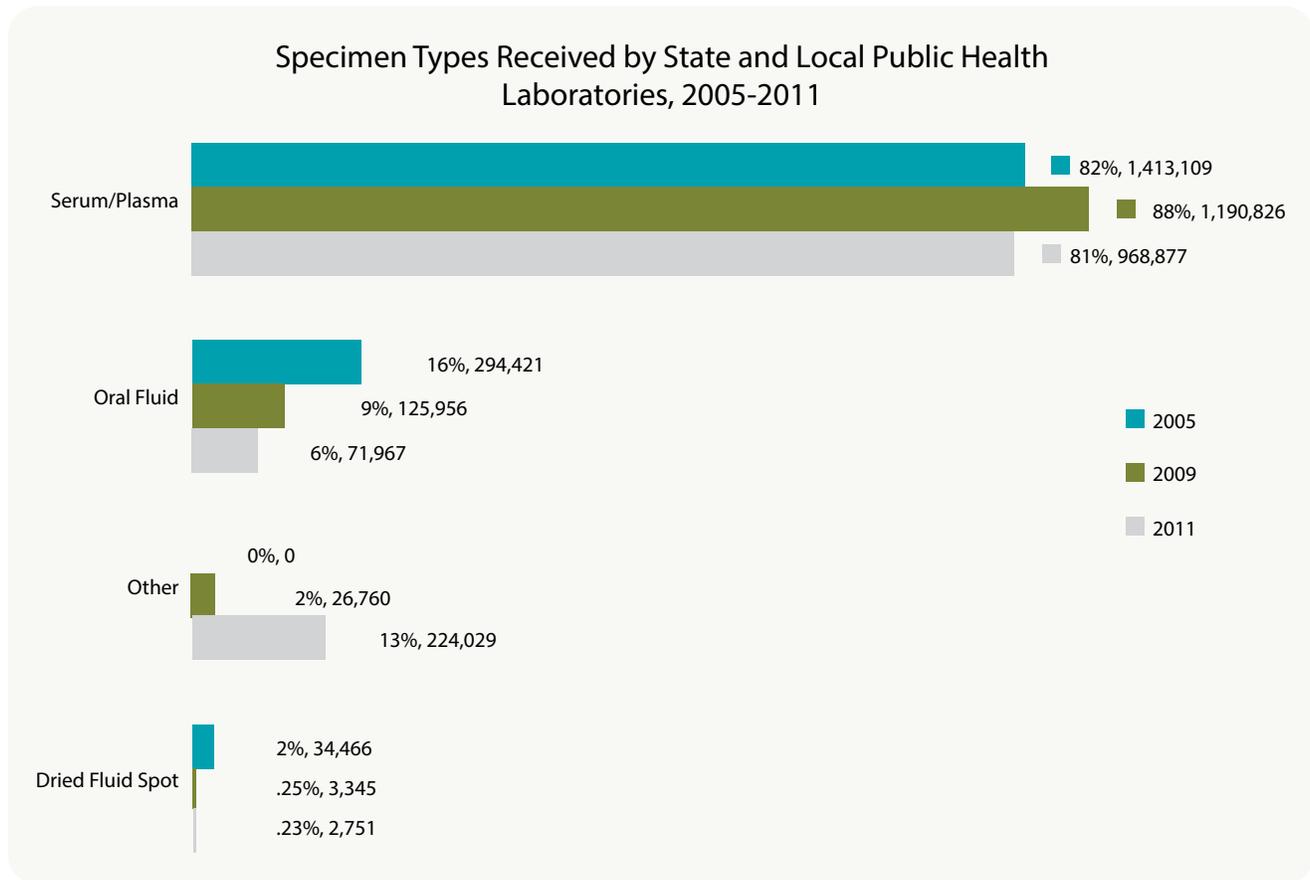


Figure 3. Comparison of specimen type percentages by total volume received by the 42 public health laboratories responding to the 2005, 2009 and 2011 surveys



IDENTIFYING PRESCREENED SPECIMENS

In 2011, 62 (95%) of the responding laboratories received specimens that were previously prescreened as reactive at point-of-contact (POC) sites. The number of prescreened specimens received by those laboratories in 2011 accounted for less than one percent (n=7,567) of the total specimens received. Only a small fraction (5%, 3) of the 62 laboratories did not know whether a specimen was prescreened as reactive at a POC site. Communications from the POC site that a specimen was identified as preliminarily positive in the field mainly came either via a checkbox on the requisition form or a handwritten note indicating the POC result.

Of the 59 respondents that receive rapid test results from the POC, 75% (44) indicated that they are aware of the type of specimen used for the rapid test. Regardless of the type of specimen tested at the POC, it appears that serum/plasma accounted for the majority of specimens sent to public health laboratories for confirmatory testing. APHL asked laboratories about the results that were reported on specimens received that were

prescreened by rapid tests. However, only 43 of the 59 laboratories performing supplemental testing on prescreened specimens were able to provide this information. Table 1 displays HIV test results reported on 5,606 (74%) of the prescreened POC reactive specimens.

While the majority of specimens (n=4,402, 79%) prescreened by rapid test as reactive at the POC had results that were reported as positive by the laboratory, there were a large number of results obtained in the public health laboratory that did not agree with the reactive rapid test results obtained at the POC on the same specimen. Nearly 17% (732) of the serum and plasma specimens received in the laboratory after a reactive rapid test result were reported as negative, indeterminate, or inconclusive. Oral fluid specimens received after a reactive rapid test result demonstrated even poorer concordance; 37% of these specimens had results reported as negative (26%), indeterminate (10%) or inconclusive (1%).

Table 1. Results reported from 43 public health laboratories that performed supplemental testing on oral fluid and serum/plasma specimens that had a reactive rapid test at the POC

HIV Test Results Reported on Specimens Prescreened as Reactive at the POC						
	Negative	Antibody Positive	Indeterminate	Inconclusive	Not Tested	Total
Serum/ Plasma	625	3,668	82	25	17	4,417
Oral Fluid	308	734	116	9	22	1,189

WORKFORCE

APHL also inquired about workforce in the HIV laboratory. In the 65 laboratories that responded in 2012, an average of 2.4 full-time equivalents (FTEs) were employed for HIV testing. The lowest number of FTEs employed at a single laboratory was 0.2 and the highest was 10.

The subset of 42 laboratories that responded to previous HIV testing surveys reported employing an average of 2.8 and a range of 0.2-10 FTEs for HIV testing in 2012 compared to an average of 3.2 and a range of 0.5-12 FTEs in 2009.

HIV SERUM/PLASMA TESTING STRATEGIES

Sixty (92%) of the 65 respondents routinely utilize one antibody test for initial screening of HIV specimens, while only five (8%) use alternative screening algorithms. Of those five, three laboratories utilize two antibody tests sequentially; if the initial antibody assay is reactive, a second different antibody test is performed. The remaining two respondents conduct parallel (or tandem) testing; all specimens are screened with two different antibody tests.

Of the 60 responding laboratories that use a single IA as the initial test in their HIV algorithm in 2012, 36 (60%) use the Bio-Rad GS HIV-1/HIV-2 Plus O EIA, a third-generation IA; while 6 (10%) use a third-generation IA other than Bio-Rad; 9 (15%) use Abbott Architect HIV Ag/Ab Combo, a fourth-generation IA; 4 (7%) use the Bio-Rad GS HIV Combo Ag/Ab EIA, a fourth-generation IA; 4 (7%) use Avioq HIV-1 Microelisa, a second-generation IA; and one laboratory used a rapid test.

The landscape of IAs used as initial screening tests by public health laboratories continues to evolve as a new generation of tests becomes available and others are withdrawn from the market (see Table 2). In 2004 and 2005, the majority of public health laboratories utilized a first-generation enzyme immunoassay (EIA) (bioMérieux Vironostika HIV-1 Microelisa system). By mid-2009, the majority (75%, 39) of laboratories using a single IA reported utilizing a third-generation EIA [Bio-Rad (Genetic Systems) HIV-1/HIV-2 Plus O]. In the 2012 survey, the percentage of laboratories utilizing a single IA reported using a third-generation EIA decreased to 70% (n = 42). Twenty-two percent (n=13) of respondents are currently utilizing a fourth-generation assay for HIV screening, and 7% (n=4) are performing screening with the Avioq HIV-1 Microelisa System; 2% (n=1) used a rapid test as the initial test in their algorithm.

Public health laboratories are beginning to move away from the WB and IFA for supplemental testing. Although the majority of (80%, n=52) respondents are still utilizing either a WB (66%, n = 43) or IFA

(13%, n= 9) for supplemental testing, this is much lower than in 2009, when all public health laboratories indicated that they were still utilizing either a WB or IFA. The Multispot HIV-1/HIV-2 Rapid Test, the supplemental test method recommended in the proposed algorithm, is being utilized by 9% (n=6) of respondents to the 2012 survey. Five (8%) respondents refer specimens to another laboratory for supplemental testing, and two (3%) use an HIV NAT assay as their supplemental test.

Discordant screening and supplemental results (e.g., EIA+/supplemental test negative) are problematic, especially when a laboratory does not have access to additional tests to help clarify the diagnosis. Fortunately, this does not occur often (less than 0.25% of specimens received in 2011 had discordant results); however, 43% (n=26) of respondents were able to perform additional testing to help resolve the discordance.

Discordant results between screening and supplemental test may occur due to HIV-2 infection. Of the 26 public health laboratories that routinely conduct additional testing on specimens with discordant results, 25 (96%) perform HIV-2 testing, with 17 (65%) utilizing the Multispot HIV-1/HIV-2 Rapid Test, 6 (23%) using an HIV-2 IA, and 2 (8%) using an HIV-2 WB. The use of an HIV-1/HIV-2 differentiation assay, like the Multispot, can help identify potential cases of HIV-2 infection, since there are no FDA-approved HIV-2 WB or IFAs available in the United States.⁶ However, only 8 (47%) of those laboratories utilizing the Multispot HIV-1/HIV-2 Rapid Test as a supplemental assay actually report out an HIV-2 positive result if the rapid test demonstrates reactivity to HIV-2 only. Eleven (65%) respondents utilizing the Multispot HIV-1/HIV-2 Rapid Test refer these specimens to the CDC or their state public health laboratory for further consultation and testing. Two laboratories report HIV-2 results and send the specimen to CDC for additional testing. This reluctance to report positive HIV-2 results may be due to the fact that there are currently no guidelines or recommendations on how to report such results.

Table 2: HIV immunoassay utilization for screening serum/plasma specimens (number of laboratories using each assay by survey year). Note: This figure shows only those assays used by laboratories using only one immunoassay for initial HIV screening

Serum/Plasma HIV Immunoassay Utilization		2006	2009	2012
4 th Generation IA	Abbott Architect HIV Ag/Ab Combo			9 (15%)
	Bio-Rad GS HIV Combo Ag/Ab EIA			4 (7%)
3 rd Generation IA	Bio-Rad GS HIV-1/HIV-2 Plus O EIA	9 (15%)	39 (75%)	36 (60%)
	Abbott HIV AB HIV 1/2	7 (12%)	2 (4%)	1 (2%)
	ADVIA Centaur HIV 1/O/2 Enhanced		3 (6%)	3 (5%)
	Ortho VITROS Anti-HIV 1+2 Immunoassay		0 (0%)	2 (3%)
2 nd Generation IA	Bio-Rad Plus rLAV EIA	7 (12%)	5 (10%)	
	Avioq HIV-1 Microelisa			4 (7%)
1 st Generation IA	bioMérieux Vironostika HIV-1	37 (62%)		
3 rd Generation Rapid Test	Uni-Gold Recombigen HIV			1 (2%)
2 ND Generation Rapid Test	Clearview STAT-PAK Assay		2 (4%)	
	OraQuick ADVANCE		1 (2%)	

TRENDS IN ORAL FLUID TESTING

The FDA approved the Avioq HIV-1 Microelisa System, a second-generation assay, in September 2009, making it the only FDA approved laboratory-based IA for oral fluid that is currently commercially available in the US. This qualitative IA detects HIV antibodies in human serum, plasma, dried blood spots and oral fluid specimens.⁷ After the withdrawal of the bioMérieux Vironostika HIV-1 Microelisa system for oral fluid screening in 2007, many public health laboratories performed a validation of the serum/plasma-based Bio-Rad (GS) HIV-1/HIV-2 Plus O immunoassay for off-label use with oral fluids.

Of the 32 public health laboratories that reported conducting oral fluid testing in 2012, 44% (n=14)

utilize the FDA-approved Avioq HIV-1 Microelisa System, while 41% (n=13) still utilize the Bio-Rad (GS) HIV-1/HIV-2 Plus O immunoassay off-label. Two of the remaining respondents indicated that they utilize a fourth-generation antigen-antibody assay to screen for oral fluid specimens. The remaining 3 laboratories use the OraQuick Advance rapid test. Of the 32 respondents that screen oral fluid specimens, 24 (75%) utilize the OraSure HIV-1 WB to confirm those results. Six of 32 (19%) respondents request a serum specimen if the oral fluid screen is reactive and two (6%) laboratories send specimens to a reference laboratory for the oral fluid WB.

HIV NUCLEIC ACID TESTING PRACTICES

The use of NATs remained relatively consistent since the previous APHL survey. In 2012, 21 (32%) of respondents reported the use of an HIV NAT platform in their laboratory, compared to 19 (31%) in 2009. One-third (34%, 15) of the 44 public health laboratories that do not perform an HIV NAT assay in-house, refer specimens to other laboratories. Most of these respondents send specimens to another public health laboratory (53%, 8), a commercial laboratory (i.e., Quest or LabCorp) (33%, 5), or CDC (20%, 3).

Of the 21 public health laboratories that perform HIV NAT in-house, 8 (38%) indicated that they perform molecular testing for clinical management of HIV-infected individuals, and 7 (33%) state that they test for acute HIV infections, and 6 (28%)

use NAT for both clinical management and identifying acute infections. Eight (38%) respondents indicated utilizing NAT to resolve discordant (i.e., EIA+/WB-) results. With regards to acute HIV infection testing, 7 (11%) respondents pool specimens to screen seronegative samples. Pool sizes range from 10 to 256 specimens.

Seven (16%) public health laboratories indicated their intention to implement HIV NAT in the next 12 months. All 7 indicated that they plan to implement a qualitative assay. Twenty seven (61%) of the 44 public health laboratories not offering NAT have no intentions to bring on a molecular-based testing platform for HIV, and the remaining 10 (23%) respondents are unsure.

ADOPTION OF NEW TESTING METHODS

The Association inquired about the uptake and implementation of the new fourth-generation IAs in public health laboratories. Twenty-four (37%) respondents have a fourth-generation assay in their laboratory. Twenty-one of those laboratories are state public health laboratories, and 3 are local public health laboratories. Thirteen (20%) respondents are currently reporting results from this new platform. Another 10 (15%) public health laborato-

ries were still completing a validation at the time the survey was conducted. Additionally, 22 (34%) respondents planned on implementing a fourth-generation assay in the next 12 months. Thus, within the year, 46 (71%) of reporting public health laboratories should be using a fourth-generation IA.

IMPEDIMENTS TO ADOPTING NEW TECHNOLOGIES AND ALGORITHMS

With the introduction of new technologies and HIV testing strategies, public health laboratories are at the forefront of implementing new diagnostic strategies and diagnostic testing algorithms. However, many factors are involved in employing new technology, especially in publically-funded organizations. This survey inquired about specific impediments for public health laboratories that hinder their ability to implement new technologies and strategies. Respondents were surveyed regarding fourth-generation antigen-antibody testing platforms and introduction of new supplemental tests including implementing HIV-1/HIV-2 differentiation assays.

Not surprisingly the greatest reported impediment to implementing a fourth-generation HIV testing platform was related to the cost. Eighteen (47%) of the 38 laboratories that have not yet implemented the assay cited cost as the top impediment. Other obstacles for bringing on a fourth-generation assay included no perceived need for such testing in the laboratory's jurisdiction (13%) and low volume of testing (13%).

When asked to rank impediments to implementing a supplemental test other than the WB or IFA; 32 of the 51 (63%) laboratories still using those assays cite the lack of formal recommendations as an impediment, making it the most common impediment noted. However, the impediment most com-

CONCLUSION

The data from the 2012 HIV Diagnostics Survey reveal several trends in public health laboratory testing practices since the 2009 HIV Diagnostics Survey was conducted. Overall testing volume in public health laboratories, as well as the volume of oral fluid specimens, continues to decline. Some of the decrease in overall volume may be attributed to the uptake in POC rapid tests since the 2005 HIV Diagnostics Survey. Another possible reason could be the shift of HIV screening to private laboratories allowing limited public health dollars to be used for more specialized tests. However, public health laboratories continue to play an important role in confirming results of specimens originally tested at the POC.

In addition to the decline in testing volume, the survey data also demonstrate a decrease in workforce dedicated to HIV testing. The decrease in FTEs may be related to the decrease in HIV testing volume or

monly ranked as number one (but cited by fewer laboratories than lack of recommendations) was cost, with 16 of the 51 (31%) laboratories ranking cost the number-one impediment. Workforce (22%) and Clinical Laboratory Improvement Amendments (CLIA)/FDA regulatory requirements (29%) were also commonly cited as impediments to implementing supplemental testing other than WB or IFA. Fourteen (27%) laboratories noted that either state regulations requiring WB (n=9) or resistance from their jurisdiction's department of health (n=5) has led to them continuing to perform WB.

Twenty-eight laboratories ranked their greatest impediments to implementing an HIV-1/HIV-2 antibody differentiation assay as a routine supplemental test. Cost/funding was the most frequently cited challenge (75%) followed by workforce (25%) and a lack of perceived need (25%). These impediments highlight the continued and ongoing resource and budget challenges faced by public health laboratories today. Despite the advent of new technologies and strategies to identify HIV infection, without proper financial stability and infrastructure, implementation of newer technologies will continue to be a struggle.

It may be representative of the overall decrease in public health laboratory workforce that has been observed in recent years. The Association will continue to monitor overall public health laboratory workforce trends.

The 2012 survey data show that several public health laboratories are identifying acute HIV infections on their test reports. This practice may become more common as more laboratories begin to adopt the proposed HIV diagnostic testing algorithm. Public health laboratories are demonstrating rapid uptake of fourth-generation IAs, as approximately half have, or are currently in the process of implementing, the assays. Costs associated with implementing a new assay is noted as a major barrier for those laboratories that are not in the process of implementing fourth-generation IAs.

It appears that the adoption of supplemental tests other than WB or IFA has been a more challenging element of the proposed algorithm to implement. Although a small percentage of laboratories have moved away from WB or IFA, the majority have not. In addition to funding issues, many of those laboratories cite the lack of formal recommendations to be a major barrier to moving away from the WB. Other commonly noted impediments including state regulations that require WB or IFA and resistance to implementing a different assay within their jurisdiction, may also be eased by the release of formal recommendations. CLIA and FDA regulatory requirements were also frequently noted impediments potentially due to the difficulties of HIV-2 validation

of the Multispot or the absence of language in the Multispot package insert indicating that it cannot be used as a confirmatory assay, which is seen as problematic in many laboratories.

APHL and CDC will continue to explore opportunities to assist public health laboratories in adopting the proposed HIV diagnostic testing algorithm and to evaluate its performance in public health laboratories as that continues to happen. APHL will continue to work with federal, state, local and non-governmental partners to address HIV testing challenges and improve HIV testing practice in the country's public health laboratories.

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