

# HIV: 2009 DIAGNOSTIC SURVEY

## FACING NEW CHALLENGES

Twenty years ago, the Association of Public Health Laboratories (APHL) and the Centers for Disease Control and Prevention (CDC) developed the first testing recommendations for the diagnosis of HIV infection. These recommendations utilized the latest technologies and best testing practices of the time.

Since the development of the first HIV testing algorithm, HIV testing technology has changed significantly with the introduction of new immunoassays, point-of-care rapid tests and molecular detection techniques. While revisions to the HIV testing guidelines have occurred periodically, the recommended HIV diagnostic algorithm has remained largely unchanged since its introduction in 1989.

Updating the HIV testing guidelines is a priority for both APHL and CDC. In the spring of 2009, APHL and CDC published "HIV Testing Algorithms: A Status Report." This report outlines the current state of potential testing algorithms proposed by APHL-CDC working groups in 2008, and identifies key data that are still needed to evaluate their performance. These proposed algorithms were designed to address many of the changes, improvements and challenges that have arisen in HIV diagnostics since the development of the first HIV testing algorithm. For example, detection and confirmation of acute and early HIV infection is an ongoing challenge

to laboratories. Western Blot (WB) and indirect immunofluorescence assay (IFA) are typically unable to detect antibodies for the first four to eight weeks, a gap known as the "window period."<sup>1</sup> Many third generation immunoassays are more sensitive during early infection than the WB and IFA, leading to discordant and/or indeterminate results. Another challenge that laboratories face is the detection of HIV-2. While many immunoassays available today are able to detect and even differentiate this viral type, their use is not universal, and there is currently no FDA-approved confirmatory test available in the US. Finally, nucleic acid amplification testing (NAAT) has enabled viral detection during the first weeks of infection, but the cost and complexity of this test have limited its adoption.<sup>2</sup>

APHL periodically surveys its members to assess their HIV diagnostic testing capabilities, capacities and practices.<sup>3</sup> Past surveys have tracked changes in the types of specimens laboratories receive, the test kits they use and the technologies they implement. Since the last survey, conducted

in 2006, there have been significant changes in the landscape of HIV testing including FDA approval of the first diagnostic nucleic acid amplification test (NAAT), withdrawal from the market of the only FDA-approved immunoassay for screening oral fluid specimens, and the continued increase of HIV screening in non-laboratory settings.

In order to learn how public health laboratories have met these challenges and to better understand the barriers faced in implementing new testing strategies, APHL launched the 2009 HIV Testing Practices Survey in July 2009. This issue brief summarizes the results of the survey.

## Specimen Types Received by State and Local Public Health Laboratories

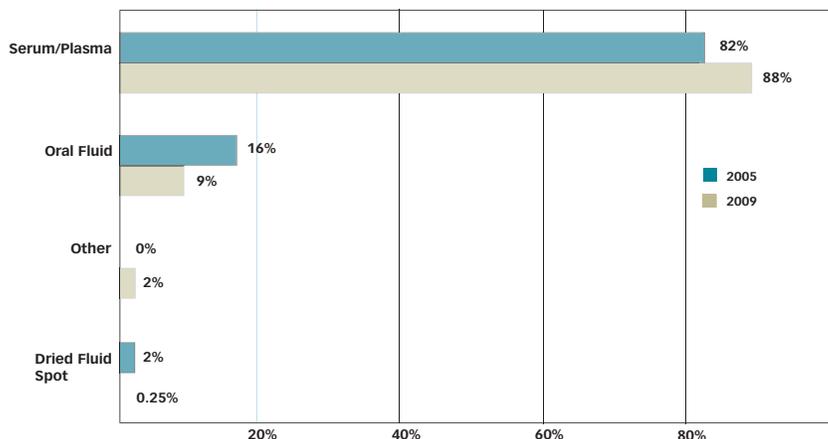


Figure 1. Comparison of specimen types received by public health laboratories as percentage of total specimen load reported in 2006 and 2009.

### METHODS

In July 2009, APHL conducted a survey to determine the HIV testing practices, capacities and capabilities of the country's state and local public health laboratories. This 23-question survey was developed by the members of the APHL/CDC HIV Steering Committee and administered through MRInterview, a web-based survey instrument. Unless otherwise specified, "respondents" refers to all survey respondents that conduct HIV testing. Survey questions were grouped into the following categories: HIV Testing Volume, Serum/Plasma Testing Algorithm, Oral Fluid Testing Practices, HIV-2 Testing, and

Use of Nucleic Acid Testing Technology. Respondents were asked to provide data reflecting the status of HIV testing in their laboratory from July 1, 2008, through June 30, 2009.

Eighty-three public health laboratories received the survey, and 69 (83%) responded: 42 of 50 (84%) state laboratories, and 27 of 38 (71%) local laboratories. In total, 61 (42 state, 19 local) respondents indicated that their laboratory conducted HIV testing at the time of the survey.

Data from the 2009 HIV Testing Practices survey were compared to data from past surveys to observe trends in public health laboratory HIV testing practices. The 2006 HIV Diagnostics Survey provided HIV testing data for 2005, and the 2004 HIV Diagnostics Testing Utilization Survey gave a snapshot of testing practices in July 2004. To evaluate changes in HIV testing volume, data were compiled for the subset of laboratories that completed both the 2006 and 2009 surveys (12 local and 38 state public health laboratories).

### SPECIMEN TYPES AND TESTING VOLUMES

From July 1, 2008, to June 30, 2009, respondents received 1,685,112 specimens for HIV testing. Over 88% of the specimens tested during this period were serum or plasma, and 9% were oral fluid specimens. This represents a considerable decrease in oral fluid specimens, which accounted for 16% of specimens in 2005 (Figure 1). To evaluate changes in testing volume more closely over time, data were compiled for the subset of laboratories that completed both the 2006 and 2009 surveys (12 local and 38 state public health laboratories). For these 50 laboratories, total specimen volume decreased by 22.2% between 2005 and 2009. The number of oral fluid specimens received for testing decreased by 53.5% over the same period. In fact, 10 laboratories (two local and eight state PHLs) stopped receiving oral fluid specimens between 2005 and 2009, which contributed to this sharp decline. The

decrease in oral fluid specimens does not account for the total drop in testing volume, however, and a general downward trend in HIV testing performed by PHLs can be seen (Figure 2).

Since the arrival of CLIA-waived rapid testing, HIV screening in non-traditional settings (such as community-based organizations and mobile testing units) has steadily increased. Public health laboratories, however, maintain a critical role in the confirmation and reporting of infection. Respondents confirmed 28,311 infections, which represents a positivity rate of 1.68%. For comparison, the rate for specimens tested in 2005 was 1.44%. It is plausible that increased prescreening at the point-of-care (POC) contributed to the increased positivity rate for specimens tested in public health laboratories.

While POC testing has increased, many public health laboratories do not receive information about the number and type of tests conducted and the results obtained in those settings. In 2009, as in 2006, the survey indicates that less than one percent of the specimens received (9,013 total) had been previously screened by rapid test. However, subsequent questioning shows that 28% of laboratories are unaware what tests have been previously conducted, so this number may be much higher than reported. This fact highlights the need for the development of specific communication systems between public health programs, clinical settings and public health laboratories that allow for reliable identification of tests previously conducted on a given specimen including flagging specimens that have previously screened positive.

Of the specimens known to have come from patients prescreened as reactive at the point of care, 41% (4,658) were initially tested using oral fluid. Respondents only received 2,419 oral fluid specimens, however, indicating that many testing sites collect a blood specimen following a reactive oral

fluid rapid test. Indeterminate results were reported for 3.5% of prescreened oral fluid specimens and 2.4% of prescreened serum or plasma specimens. Inconclusive (i.e., EIA+/WB-) results were reported for 4.1% of prescreened oral fluid specimens and 1.1% of serum or plasma specimens. Based on these data, specimens screened as oral fluid were much more likely to yield indeterminate or discordant results than serum or plasma specimens.

## HIV Testing Volume Trends for PHL System, 2005-2009

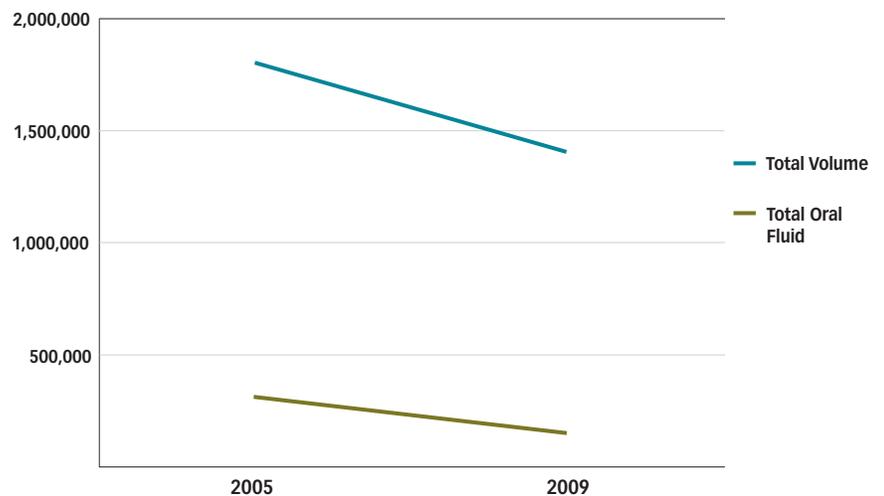


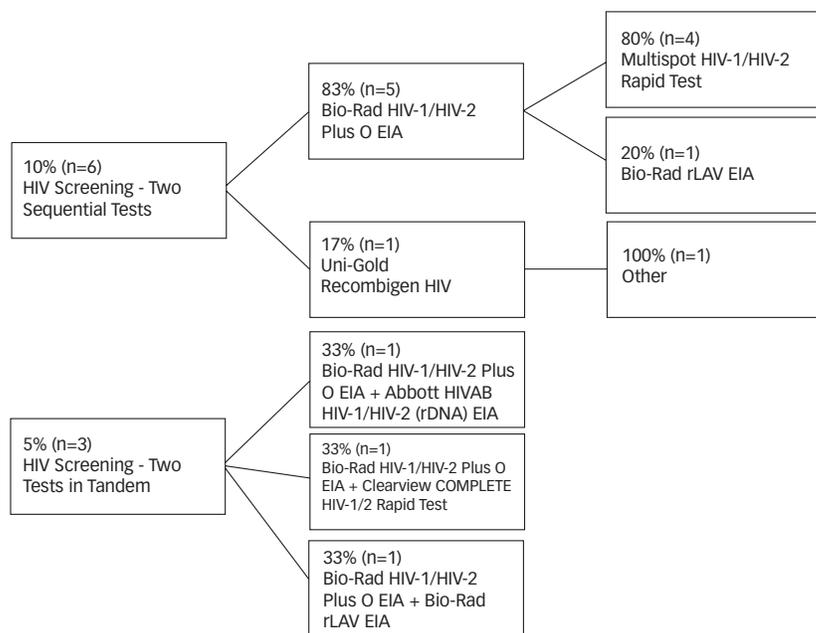
Figure 2. Comparison of specimen volume for laboratories that completed both the 2006 and 2009 APHL HIV surveys.

## HIV TESTING ALGORITHMS FOR SERUM & PLASMA SPECIMENS

As in previous years, HIV testing practices very closely followed the traditional APHL/CDC guidelines for HIV diagnosis: a specimen repeatedly-reactive by an initial immunoassay is confirmed via a positive supplemental test (a WB or an IFA). Fifty-two of the 61 respondents (85%) routinely use only one screening test while nine laboratories (15%) use alternative screening algorithms. Of those nine, three laboratories conduct parallel (or tandem) testing; that is, all specimens are tested with two different immunoassays. The other six laboratories screen with multiple immunoassays sequentially; if the initial immunoassay is positive, a second immunoassay--usually an HIV-1/2 discriminatory assay--is conducted (Figure 3).

The immunoassays used as initial tests by public health laboratories have changed as tests have been added to and withdrawn from the market (Figure 4). In 2004 and 2005, most laboratories (61% and 64% respectively)

### Multi-Test HIV Screening Strategies Utilized by Public Health Laboratories, 2009



**Figure 3: Assays used in multi-test HIV screening algorithms in public health laboratories between July 1, 2008 and June 30, 2009.**

utilized the bioMérieux Vironostika HIV-1 Microelisa (a first generation enzyme immunoassay). In the 2009 survey, 77% of respondents (47 laboratories) report using the Bio-Rad (Genetic Systems) HIV-1/HIV-2 Plus O EIA as part of their HIV screening algorithm, either in a one or two test algorithm; this represents a significant increase from the 16% of laboratories that reported using the assay in 2005.

The survey results verified that all public health laboratories utilize HIV confirmatory tests that reflect the traditional HIV testing algorithm. Of the 57 laboratories that conduct confirmatory testing on serum and plasma specimens, 51 respondents (89.5%) utilized WB as their main confirmatory testing while six (10.5%) used IFA. Although the majority of respondents used only one test during confirmatory testing, a third of HIV-confirming laboratories (19

of 57) indicated that they routinely used additional tests to resolve an indeterminate WB or IFA. The types of additional testing used to resolve indeterminate results include: Multispot discriminatory HIV-1/HIV-2 immunoassay (n=17, 30%), NAAT (n=10, 17.5%), HIV-2 EIA (n=3, 5.3%). Of the seven laboratories using IFA as their primary confirmatory test, two used WB to resolve an indeterminate result. Of the 51 laboratories that use WB as their main confirmatory test, one used IFA to resolve indeterminate test results. Additionally, 82.5% (47) of HIV-confirming laboratories will request a follow-up specimen and 26% (15) will send specimens to a reference laboratory for additional testing.

Many laboratories also use additional testing when the screening and confirmatory test results are discordant (e.g. EIA+/WB-). Of the 57 laboratories that conduct confirmatory serum/plasma testing, 14 (24.6%) use the Multispot discriminatory HIV-1/HIV-2 immunoassay, 14 (24.6%) refer the specimen for HIV-2 testing, and six (10.5%) conduct NAAT for acute infection. Thirty-two laboratories (56.1%) will request a follow-up specimen, and three will send the specimen to a reference laboratory for additional testing.

### TRENDS IN ORAL FLUID TESTING

Public health laboratories began accepting oral fluid specimens for initial screening and confirmatory testing in response to programmatic requests for a noninvasive means of collecting specimens for testing in outreach settings. With the approval in March 2004 of the first rapid test for oral fluid screening in CLIA-waived testing sites, public health laboratories began receiving a number of prescreened-reactive oral fluid specimens for confirmatory testing as well. In 2007, bioMérieux withdrew the Vironostika HIV-1 assay from the market, leaving the market void of an FDA-approved oral fluid screening assay during the time span that this survey covered. Public health laboratories that wished to continue testing oral fluid were required to

establish the performance characteristics of an immunoassay approved for serum/plasma testing for off-label use on oral fluid specimens. During this time, many laboratories implemented the BioRad (GS) HIV-1/HIV-2 Plus O Immunoassay, a third generation EIA. It should be noted that in September 2009, the Avioq HIV-1 Microelisa System received FDA approval for the qualitative detection of antibodies in oral fluid specimens.

Fifty-eight percent of respondents to the 2004 HIV Diagnostic Testing Utilization Survey conducted oral fluid testing. By 2006, that had increased to 70.3%. As of 2009, this percentage has dropped, with only 30 survey respondents (49.1%) indicating they still conduct oral fluid testing. Of those 30 respondents still conducting HIV testing on oral fluid, 86.7% now use the Bio-Rad (GS) HIV-1/HIV-2 Plus O immunoassay as their initial test. Of the remaining laboratories, one laboratory uses the Bio-Rad (GS) HIV-1 rLAV assay, one uses the OraQuick ADVANCE Rapid HIV-1/HIV-2 Antibody Test, and two laboratories don't perform any primary screening on oral fluid specimens (only conducting the OraSure HIV-1 WB confirmatory test).

## HIV-2 TESTING PRACTICES

In 2006, approximately one-third of public health laboratories screened with an assay approved for detecting HIV-2; today, over 90% of survey respondents used an HIV-1/HIV-2 screening assay in their diagnostic algorithm. HIV-2-positive specimens will often test indeterminate or negative during traditional HIV-1 confirmatory testing (WB or IFA). While most laboratories (77%) will request a follow-up specimen in this situation, there remain few other options in the current algorithm for resolving a potential HIV-2 infection. Seventeen respondents (27.9%) have begun using the Multispot discriminatory HIV-1/HIV-2 rapid test to resolve these indeterminate or discordant (immunoassay-positive/confirmatory test-negative) results. Fifty-five percent of laboratories send specimens

to CDC for further testing. CDC uses a number of methods to confirm HIV-2 infection.

Five (10%) of the responding laboratories have the ability to confirm HIV-2 infection in-house. These laboratories have developed their own testing algorithms, which include use of commercially available HIV-1/HIV-2 discriminatory assays as well as

## Multi-Test HIV Screening Strategies Utilized by Public Health Laboratories, 2009

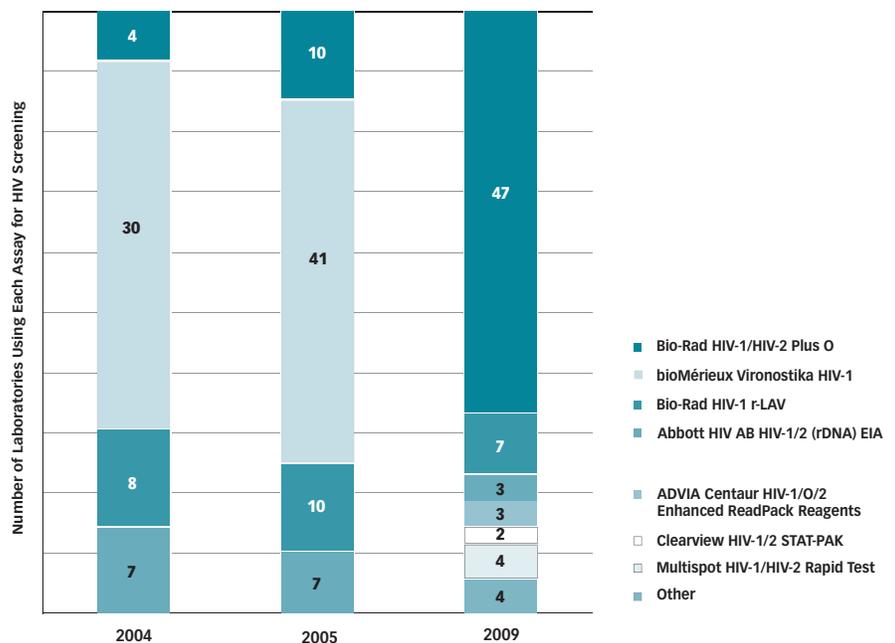


Figure 4: HIV immunoassay utilization for screening serum/plasma specimens (number of laboratories using each assay by survey year). Note: This figure shows all immunoassays routinely used by laboratories in their HIV screening algorithms. This includes assays used solely as a second test in sequential HIV screening algorithms (like the Multispot HIV-1/HIV-2 Rapid Test).

laboratory-developed HIV-2 Western blots and nucleic acid tests. Since these tests are not FDA approved for confirmation, a laboratory must establish the performance characteristics of these assays before putting them into use.

## HIV NUCLEIC ACID TESTING UTILIZATION

Many public health laboratories have implemented nucleic acid amplification testing in recent years. These molecular techniques have a number of diverse

applications and meet several needs of public health laboratories. Nineteen survey respondents (31%) indicated that they currently perform NAAT for HIV. Thirteen laboratories (21.3%) use NAAT for viral load, and five laboratories

(8.2%) perform resistance testing to monitor for mutations that confer antiretroviral drug resistance. Ten laboratories (16%) report using NAAT to resolve indeterminate Western blots and discordant (i.e. EIA+/WB-) results. Because some immunoassays available today can be more sensitive than WB and IFA prior to seroconversion, these indeterminate and discordant results can often represent an early or acute HIV infection. Ten laboratories (16%) report performing acute infection testing on request (16.4%). Seven laboratories (11.5%) screen all seronegative specimens—or those that test negative upon initial HIV antibody screening—with pooled nucleic acid amplification testing, compared to only one laboratory that reported conducting this screening in 2005.

## IMPEDIMENTS TO IMPROVED HIV TESTING

Implementing new technologies and updated HIV testing algorithms will ensure that public health laboratories have the tools necessary to meet public health needs for HIV diagnosis and patient care. Survey respondents were asked about the impediments they face in implementing these changes. Funding concerns were by

**Funding concerns were by far the most common impediment with 43% of laboratories citing it as the greatest barrier to implementing new technologies and alternative algorithms.**

far the most common impediment with 43% of laboratories (24) citing it as the greatest barrier to implementing new technologies and alternative algorithms. Twenty-eight percent of laboratories (17) cited workforce concerns, with physical laboratory space (8%) and regulatory issues (5%) also being of concern for some respondents. Unfortunately, due to current economic constraints, some laboratories are being forced to consider dropping services like NAAT screening for acute infection. This development hinders the performance of quality HIV diagnostics and highlights the ongoing resource and budget challenges faced by public health laboratories.

## CONCLUSIONS: THE FUTURE OF HIV TESTING

The data from the 2009 HIV Diagnostics Survey provide a snapshot of current HIV testing practice in public health laboratories across the United States, and the data reveal several notable changes since the 2006 HIV Diagnostics Survey was conducted. In recent years, laboratories have confronted many challenges: testing oral fluid specimens despite the lack of an FDA-approved screening assay, reducing and resolving indeterminate and inconclusive results, screening for diverse HIV types and subtypes, supporting public health programmatic efforts in HIV testing, and implementing new testing technologies. Overall testing volume in public health laboratories has decreased since 2005 with the greatest decrease seen in oral fluid specimen volume. However, laboratories continue to play an important role in confirming HIV infection. The majority of public health laboratories now use a third generation immunoassay for screening, which has increased capacity for detecting HIV-2 and HIV-1 Group O infections. Several challenges remain, however, including obtaining adequate information regarding previous testing conducted on specimens submitted from CLIA-waived testing sites, confirming HIV-2 infections, increasing acute HIV infection screening, and improving communication and workflow

between point-of-care testing sites and laboratories.

The withdrawal from the market of the only FDA-approved oral fluid screening assay in 2007 forced laboratories to establish the performance characteristics of a serum/plasma assay if they wished to continue testing these specimens. This drove a shift to the use of a third generation immunoassay for oral fluid screening. The lack of an FDA-approved assay continued until September 2009 when the Avioq HIV-1 Microelisa System received FDA approval for use on oral fluid specimens. Consequently, in the coming months many public health laboratories will likely implement the Avioq assay, a first generation immunoassay that is not licensed for HIV-1 Group O or HIV-2 testing.

HIV testing practices in public health laboratories today largely follow the traditional CDC/APHL recommended testing algorithms, but modifications have been instituted in a limited number of laboratories. While use of nucleic acid amplification testing has increased, identifying acute cases of HIV remains

a challenge. Additionally, while several laboratories use multiple different immunoassays during preliminary screening, all still rely on a positive WB or IFA to confirm infection.

APHL and CDC proposed several alternative testing strategies in the 2009 document, "HIV Testing Algorithms: A Status Report."<sup>4</sup> These include algorithms for HIV-2 and acute HIV-1 testing, rapid testing algorithms for point-of-care sites, and use of multiple immunoassays for the laboratory diagnosis of infection. Before the proposed HIV testing strategies can be formally recommended, more data are needed to evaluate their performance. The upcoming 2010 HIV Diagnostics Conference, to be held March 24-26, 2010, will provide a forum for the evaluation of the proposed testing algorithms and discussion of the latest testing technology and other current topics (visit [www.hivtestingconference.org](http://www.hivtestingconference.org)). APHL will continue to work with federal, state, local and nongovernmental partners to address HIV testing challenges and improve HIV testing practice in the country's public health laboratories.

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## Association of Public Health Laboratories

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