

Public Health Laboratory Issues in Brief: 2006 HIV Diagnostics Survey

Association of Public Health Laboratories

November 2007

Background

Nearly 20 years ago, the Association of Public Health Laboratories (APHL) and the Centers for Disease Control and Prevention (CDC) confronted the spread of a frightening new disease by recommending an HIV diagnostic testing algorithm that utilized the best practices of the day. Today—despite the current availability of molecular technology and rapid test methods—these recommendations remain largely unchanged.

Many experts at the 2005 HIV Diagnostics Conference agreed that change is necessary to ensure laboratories use the best currently available methods for HIV diagnosis. In response, APHL's Board of Directors asked the APHL/CDC HIV Steering Committee to lead the development of new laboratory and point-of-care testing algorithms.

Original laboratory guidelines call for repeatedly reactive enzyme immunoassay (EIA) screens to be confirmed with supplemental testing such as a Western blot (WB) or immunofluorescence assay (IFA) (see www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm for the complete guidelines). New laboratory algorithms will enable options for alternative supplemental testing. Identifying HIV-2 and acutely infected individuals will also be a focus of these algorithms.

New point-of-care algorithms will provide CLIA-waived and moderately complex laboratories—such as those in community-based organizations, physicians' offices and emergency departments—with the opportunity to diagnose a client's HIV infection status on-site (for more information on the Clinical Laboratory Improvement Amendments [CLIA] of

1988, see <http://www.cms.hhs.gov/clia/>). Currently all of these testing sites must send a supplemental specimen to a laboratory for confirmation when a rapid test is reactive.

Methods

In September 2006 APHL conducted a web-based survey to assess HIV diagnostic testing capabilities, capacities and practices of public health laboratories. One hundred local, state and territorial public health laboratories were approached: respondents included 49 of the 56 (88 percent) state and territorial public health laboratories, and 20 of the 44 (45 percent) local public health laboratories. Of the local laboratory respondents, five reported no data on HIV testing. Follow-up indicates that many local public health laboratories do not perform HIV testing. The low response rate from local labs may also be attributed to unfamiliarity with APHL's survey process. In the end, 64 laboratories contributed to the data set.

Data from a 2004 HIV Diagnostic Testing Utilization Survey has also provided the APHL/CDC HIV Steering Committee with useful information and a solid basis of comparison (see http://www.aphl.org/programs/infectious_diseases/hiv/Documents/hiv_survey_report.pdf). "Respondents" when used in conjunction with survey data will refer to all public health laboratories who answered a particular question, unless otherwise specified. The 25-question survey was designed and administered through a web-based survey tool. Questions were grouped into the following categories:



Public Health Laboratory Issues in Brief

8515 Georgia Ave, Suite 700
Silver Spring, MD 20910

Phone: 240.485.2745
Fax: 240.485.2700

- HIV Specimen Volume
- Effects of Rapid HIV Testing on Public Health Laboratories
- Current Algorithms for HIV Testing
- HIV-2 Screening and Confirmation Methods
- Capabilities for HIV Nucleic Acid Amplification Testing (NAAT)
- Oral Fluid Testing Issues

HIV Specimen Volume

Respondents tested 2,279,089 HIV specimens in 2005. As expected, state public health laboratories reported a much higher average (mean=41,873.61) than local facilities (mean=15,152.13). The quantity of HIV specimens tested at each site ranged from 897 to 259,621. Respondents were asked to only report specimens that did not already have a rapid HIV test performed; therefore these numbers exclude any specimens known to be prescreened with rapid HIV tests prior to reaching the laboratory.

Public health laboratories confirmed 32,855 HIV infections in 2005, a 1.44 percent prevalence rate. This is much higher than the 0.6 percent national prevalence rate reported by USAID in 2005 for adults

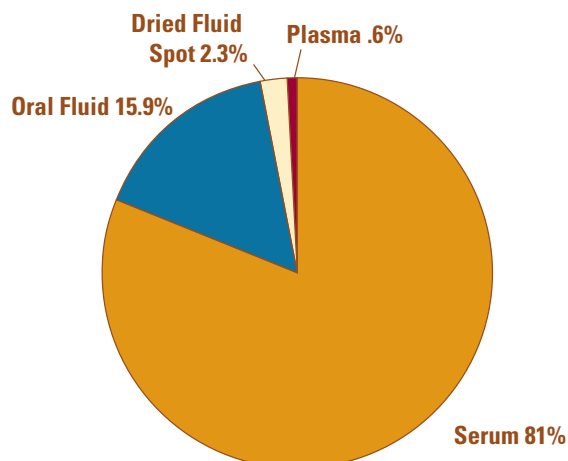
aged 15-49.⁵ The higher prevalence may indicate that public health laboratories test higher-risk populations that seek HIV testing at health departments and STD clinics. The higher prevalence demonstrates the importance of the public health laboratory's role in identifying HIV-positive individuals quickly and accurately.

Serum specimens are received most frequently, at 81.0 percent. However a significant percentage of oral fluid (15.9 percent) was also handled, highlighting the continued need for a FDA-approved EIA for this specimen type.

The survey also identified 9,253 specimens that yielded discordant testing results (EIA reactive and WB indeterminate or negative), with 61.2 percent stemming from oral fluid specimens. The survey did not capture the patient's actual health outcome for these discordant specimens, so it is unknown whether the screening or supplemental test was inaccurate. The new testing algorithms aim to reduce the number of discordant results by using newer technologies and offering more options for the algorithms to provide more accurate results. The discordant specimens identified in this survey could be helpful in testing the new strategies.

Figure 1

Percentage of Non-Prescreened HIV Specimens Received by State and Local Public Health Laboratories in 2005



Effects of Rapid HIV Testing on Public Health Laboratories

As the use of HIV rapid tests becomes more common in nontraditional laboratory settings such as STD clinics and community-based organizations, public health laboratories may see an increase in the number of specimens prescreened by rapid testing. In 2005, public health laboratories received 5,897 prescreened specimens. The majority (88.7 percent) of specimens sent to the laboratory for supplemental testing were serum or plasma; the remaining 11.3 percent were oral fluid.

Survey respondents shared the laboratory results for specimens that had been reactive by rapid test in the field. Using these data, the positive predictive

value—the probability that a positive rapid test will have a matching result from a laboratory supplemental test⁸—was calculated. Serum and plasma specimens had a positive predictive value of 94.6 percent; oral fluid specimens had a positive predictive value of 84.6 percent. These comparisons reveal a low level of disparity in accuracy among rapid tests in comparison to supplemental laboratory testing.

Less than one percent of all specimens received by public health laboratories were known to be prescreened by a rapid HIV test. However, survey follow-up revealed that some laboratories are unaware if a specimen is prescreened in the field. The increasing use of rapid HIV tests may ultimately decrease the volume of specimens received in public health laboratories. APHL will track trends in HIV testing volume through future surveys to help determine the effect of rapid HIV testing on public health laboratory volume and infrastructure.

The survey also inquired about the HIV testing algorithms public health laboratories used to confirm specimens with reactive rapid test results. Despite current CDC quality assurance guidelines that advise laboratories to perform a supplemental laboratory test (WB, IFA or nucleic acid amplification testing)^{3,7} on all reactive rapid HIV test specimens, 14 public health laboratories responded that they only perform a supplemental test, if the EIA is reactive on serum and plasma samples. There was a similar trend with oral fluid specimens; eight public health laboratories did not follow the CDC recommendations.

After follow-up with these respondents, APHL learned that some of these respondents do adhere to CDC recommendations for testing prescreened reactive specimens; but the survey question had been misunderstood. Other laboratories are not informed by the point-of-care sites when specimens have been prescreened and therefore they perform a routine algorithm that does not require a WB or IFA follow-up for a non-reactive EIA. This disconnect exposes a training need throughout the public health system, from clini-

cians and counselors who fill out the requisition forms to the laboratorians performing the follow-up testing. The APHL/CDC HIV Steering Committee will clarify the updated CDC guidelines with public health laboratories to ensure the proper tests are performed on prescreened rapid test specimens; committee members will also help improve requisition forms so laboratorians can identify prescreened rapid tests.

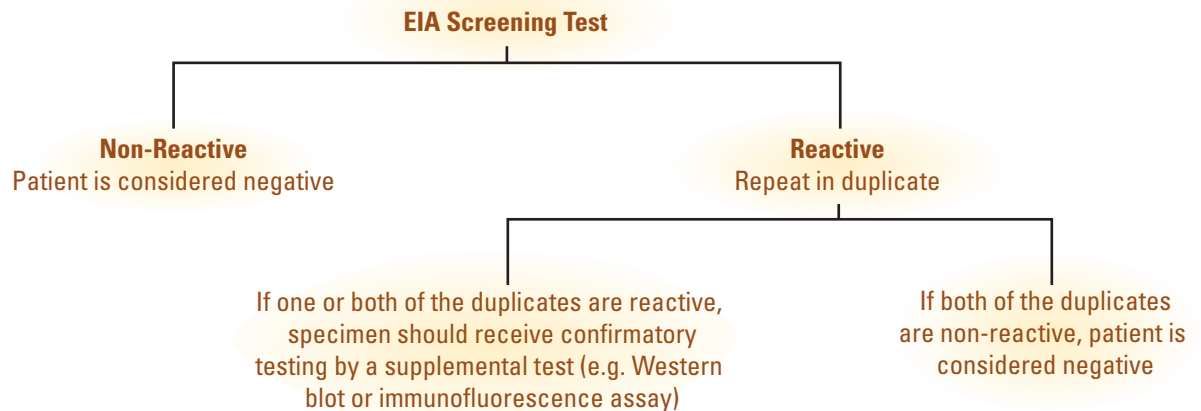
Algorithms for HIV Testing

The CDC guidelines directing laboratory practices for routine HIV screening were most recently revised in 2001. These recommendations remain largely unchanged from the original guidelines made more than 20 years ago: a screening EIA, with repeatedly reactive results checked against a follow-up WB or IFA test.⁶ Using this framework, laboratories develop localized algorithms. This survey recorded routine serum/plasma algorithms performed in 2005, as well as forthcoming changes.

Current Laboratory Screening Algorithm

Nearly all (96.9 percent) public health laboratories utilize an EIA as a screening assay (either as a primary, secondary or both in tandem). Respondents use the following EIAs as a primary screen:

- Thirty-seven (57.8 percent) used the bioMérieux Vironostika HIV-1.
- Nine (14.0 percent) used the Bio-Rad HIV-1/HIV-2 Plus O.
- Seven (10.9 percent) used the Bio-Rad HIV-1 rLAV.
- Seven (10.9 percent) used the Abbott HIV AB HIV-1/2.
- Two (3.1 percent) used two different assays simultaneously (in tandem) for the primary and secondary screens.
- Two (3.1 percent) used two different assays sequentially (a primary assay then followed by a secondary) to screen.

Figure 2 Current Recommended HIV Testing Algorithm

- Two (3.1 percent) send HIV specimens to another laboratory for supplemental testing.
- One (1.6 percent) contracts out serum/plasma HIV testing to a commercial laboratory and does not perform testing in-house.

After a primary screening assay returns reactive results, the current algorithm requires the specimen to be tested in duplicate on a secondary EIA that may or may not use the same platform as the primary EIA. Only four (6.2 percent) respondents reported using a different platform for the secondary screen. Two of these laboratories perform the primary and secondary screen on two different assays simultaneously (i.e., tandem)—these facilities may be able to provide helpful data for substantiating the new algorithms.

If one or both of these duplicate specimens is reactive during the second screen, a WB or IFA will be used for supplemental testing to confirm the diagnosis. Only seven (10.9 percent) laboratories (two state and five local) use an IFA for supplemental testing; the remaining laboratories utilize a WB. The most commonly used WB is the Bio-Rad/Genetic Systems HIV-1 WB, used by 68.8 percent of respondents. Unfortunately the current options for commercially-available supplemental testing are limited to tests that detect HIV-1 *only*; which may lead to increased num-

bers of discordant results since more laboratories are using combination screening assays that detect *both* HIV-1 and HIV-2.

From 2005 to 2006, public health laboratory testing practices remained largely unchanged. Eight additional laboratories began utilizing the Bio-Rad HIV-1/HIV-2 Plus O EIA in 2006, which in every case but one, indicated a switch from an HIV-1 only assay to a combination assay. Only two laboratories changed their confirmatory test; both laboratories switched from the Bio-Rad/Genetic Systems HIV-1 WB to either the Calypte HIV-1 WB or Fluorognost HIV-1 IFA.

A small number of laboratories (six) use various other types of assays for special circumstances. Some of these include:

- NAAT to screen newborns of HIV infected mothers
- NAAT pooling of all HIV-1 EIA negative specimens and for populations at risk for acute infection
- Rapid testing of discordant specimens

All 64 respondents followed the CDC recommendations for routine testing. Therefore the survey did not identify any public health laboratories that instituted a novel algorithm that digressed from the

CDC recommended EIA/WB or IFA strategy. However, the large number of discordant specimens (i.e., EIA repeatedly reactive with WB negative or indeterminate results) and lack of a method to identify HIV-2 infection indicate that the current algorithm is antiquated and revisions are due.

HIV-2 Detection Methods

Questions in this section refer to laboratory testing performed in 2005.

In 1986, two years after HIV-1 was characterized and isolated, a second strain, HIV-2, was discovered in Africa. HIV-2 continues to have its highest prevalence in Western Africa. Though thought to be rare in the United States, actual numbers are not available since there are no FDA-approved, HIV-2 supplemental assays. It is important to assist public health laboratories in screening and surveillance efforts for the HIV-2 strain so it does not spread undetected.⁴

About a third (34.9 percent) of respondents test for HIV-2, accounting for only about 18.0 percent of the total specimens screened nationwide. Of these 22 laboratories, the majority (63.6 percent) used the Bio-Rad HIV-1/2 plus O EIA. This low percentage could be explained by the fact that many survey respondents (70.3 percent) have maintained the use of an HIV-1 only EIA to screen specimens, as it is part of their previously established testing procedure, thus eliminating the ability to screen for HIV-2 and causing potential cases of the virus to be missed and go untreated. However due to the decreasing availability of these HIV-1 only assays, it is likely that more public health laboratories will switch to a combination screening EIA. As this happens, it will become even more important to have an algorithm that allows for confirmation of HIV-2. APHL/CDC workgroups are attempting to address this need.

The CDC serves as the national reference laboratory for public health laboratories requiring assistance with HIV-2 testing. Nearly half of the respondents (42.9 percent) send specimens from patients with

suspected HIV-2 infections to CDC for detection and confirmation. Even among the laboratories that do screen for HIV-2, the majority (59.1 percent) still send specimens to the CDC for confirmation. However, since there is currently no FDA-approved HIV-2 supplemental test, these results must be interpreted with caution since they are considered off-label testing. The CDC utilizes a discriminatory rapid HIV test (one that can distinguish between HIV-1 and HIV-2 infection) and a non-FDA licensed HIV-2 WB to confirm specimens. In addition, the CDC may use molecular methods to sequence a viral strain believed to be HIV-2 positive.

The seven public health laboratories that confirm HIV-2 infections use various assays, including non-FDA licensed WBs, discriminatory rapid HIV tests, NAATs and internally-developed molecular assays. These laboratories have identified 19 HIV-2 positive specimens, which equates to less than one percent of the total number of positive specimens. The new algorithms for HIV-2 testing will eventually provide a better picture of the nation's disease burden.

HIV Nucleic Acid Amplification Test (NAAT)

Questions in this section refer to testing performed in 2006.

Traditional screening assays such as EIAs and rapid HIV tests require sufficient amounts of HIV antibodies be present before the test can detect an infection. The time between when a person is infected and when antibodies to HIV can be detected is commonly referred to as the window period. For HIV, the window period typically lasts two to eight weeks after a person contracts the virus.

Persons in the window period are considered to be in the acute infection phase of the disease. In this stage, people are more infectious due to a higher HIV viral load.¹ Identification of these acute cases could theoretically reduce the spread of HIV by enabling public health authorities to concentrate resources in

areas with high rates of acutely infected individuals.

Unlike the traditional tests, nucleic acid amplification testing (NAAT) can identify these acutely infected individuals. Rather than relying on antibodies for detection, it detects viral RNA, which is present almost immediately after an infection is acquired.¹ Therefore theoretically NAATs allow for earlier detection.

Nearly all commercially available NAATs on the market are FDA-approved for donor screening in blood bank settings or for disease management (i.e., viral load testing). However, only one NAAT is FDA-approved for screening and supplemental testing for HIV diagnosis. The Gen-Probe APTIMA HIV-1 NAAT received FDA approval in October 2006.² This test is only approved for use with plasma specimens, greatly reducing its application since most specimens are serum. Also, the APTIMA is not currently FDA-approved for specimen pooling. Pooling, or batching specimens, is the practice of combining several small HIV plasma specimens; the test is then run on the large sample. If the batched sample is positive, the pool is deconstructed and individual units are tested to determine which are positive. This strategy can significantly reduce the cost of running the test since a high proportion of samples will be negative.

Certain factors—including HIV treatment—can lead to false negative results with NAATs; when used as a supplemental test, a negative NAAT must be followed-up with another test (such as a WB or IFA) to confirm. A NAAT can also be used in conjunction with another supplemental test to confirm a specimen that was reactive on a screening test (e.g., EIA or rapid HIV test). Nucleic acid amplification testing may also be used as a screening test. If NAATs were incorporated into a laboratory algorithm as the initial screening test in HIV diagnostic testing, it could assist with the identification of acutely positive HIV specimens that might otherwise go undetected. However, the

downside is notable: NAATs are expensive, and less than one percent of specimens are the compatible plasma type.

Forty-one public health laboratories had the equipment and reagents available to conduct HIV NAAT. However, only 16 did. Of these laboratories:

- One was using NAAT for acute infection screening.
- Five were using NAAT for resolution and confirmation of HIV despite the lack of FDA-approval for HIV diagnosis at the time.
- Five were using NAAT for screening seronegative specimens.
- Twelve were using NAAT for disease management purposes.

APHL compared the capacity of state public health laboratories performing HIV NAAT in 2003 and 2006 to mark any changes in the nation's ability to identify acute infection. (At this time, the data only encompass state public health laboratories.) Thirty laboratories have HIV NAAT capabilities, an increase from 2004 when only 25 state laboratories had capabilities.

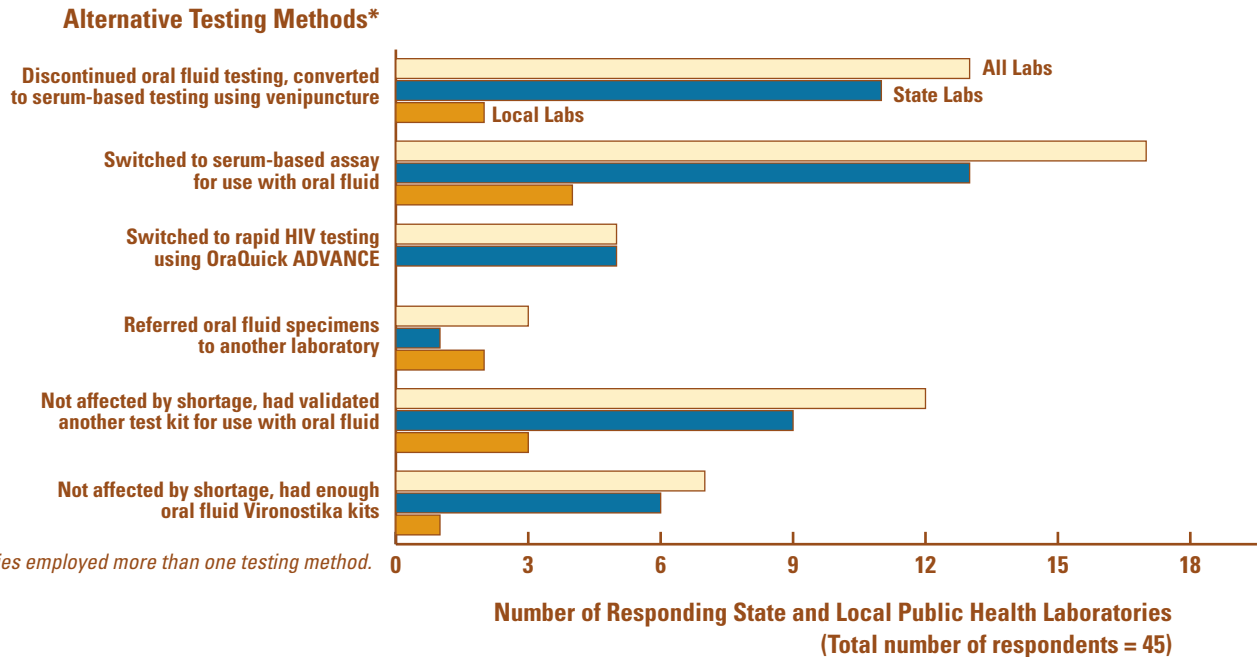
NAAT usage for acute infection screening requires further analysis to determine if the benefit will outweigh the cost. The CDC is evaluating this issue. For now APHL does not endorse use of NAAT for acute infection screening. Once more data are available, the APHL/CDC Steering Committee will evaluate the potential of this test for use in public health laboratories.

HIV Oral Fluid Testing Issues

Questions in this section refer to testing performed from February 2005 to June 2006.

In February 2005, APHL learned of supply problems for the only FDA-approved HIV oral fluid (OF) EIA kit and quickly notified its membership. As the supply of these oral fluid kits fluctuated over the next two years, APHL continued to monitor the situation.

Figure 3 Alternative HIV Testing Methods Adopted by State and Local Public Health Laboratories During the Vironostika Oral Fluid Kit Shortage in 2005



In December 2006 bioMérieux, the manufacturer, announced it would withdraw from the HIV and Human T-cell Lymphotropic Virus (HTLV) micro-plate immunoassay market by the end of 2007. This decision will end production of the Vironostika HIV-1 EIA screening platform for serum and oral fluid. In an effort to gauge the impact of this decision, the APHL survey sought more information on oral fluid testing practices in public health laboratories.

During this time, there were 45 public health laboratories, including 36 state facilities, that performed HIV oral fluid testing. Most respondents (79.5 percent) reported that the oral fluid kit shortage had some impact on their laboratory. Nearly half (48.4 percent) of these laboratories indicated that the impact was either large or very large.

Most respondents had to use alternative testing methods in response to the oral fluid kit shortages. Some of these methods included:

- Switching to a serum-based assay to test oral fluid specimens (17 laboratories);
- Discontinuing oral fluid testing and converting sites to serum-based testing using venipuncture (13 laboratories);
- Switching to an oral fluid rapid HIV test (5 laboratories).

Not all respondents had to modify existing testing practices because of the shortage: 12 labs had previously validated another serum-based test kit for use with oral fluid, and an additional seven had an ample supply of oral fluid EIA kits to endure the shortage.

Unfortunately, many (68.2 percent) of the labs that switched to a serum-based assay to test oral fluids chose the bioMérieux Vironostika HIV-1 serum assay. bioMérieux’s decision to stop production in the United States means these laboratories must find yet another option for oral fluid testing.

Many (68.2 percent) of the laboratories that

switched to a serum-based assay to test oral fluids have completed a full-scale validation; yet only 38.4 percent of these respondents have had a Clinical Laboratory Improvement Amendments (CLIA) of 1988 inspection while using an alternative assay off-label validated for oral fluid. In October 2005, APHL requested approval from the Centers for Medicare and Medicaid Services (CMS) for public health laboratories to perform small scale validations to use the bioMérieux Vironostika serum kit to test oral fluid specimens. CMS accepted the proposal, which helped more than 60 percent of the survey respondents through the shortage. In October 2007, CMS accepted a proposal from APHL allowing public health laboratories to switch to another serum based assay for oral fluid testing.

Conclusions

The survey data show that public health laboratories have been adhering closely to the currently recommended HIV testing algorithm. However, despite the numerous technological advances in HIV diagnostics, this algorithm has remained unchanged for more than 20 years. With the many novel HIV diagnostic platforms that have entered the market in recent months, it is likely that public health laboratories are now exploring options that were not available in 2005. The market will continue to change with the arrival of a fourth generation antigen/antibody capture immunoassay, currently pending FDA approval.

Workgroups established by the APHL/CDC HIV Steering Committee have developed draft strategies that will require data to substantiate performance and utility. These new algorithms offer alternatives to the standard practices of laboratory and point-of-care testing. These options include the use of new diagnostics, strategies for HIV-2 and acute infection testing and the use of multiple rapid HIV tests at the point-of-care. Ideally these strategies will be used widely in the public health, clinical and commercial arenas. These strategies, the introduction of new diagnostic tools

and other current issues will be discussed in Atlanta at the APHL co-sponsored 2007 HIV Diagnostics Conference, December 5-7, 2007 (visit www.hivtestingconference.org).

The survey also demonstrated the need for continued education, communication and collaboration among public health laboratories and federal partners. It remains essential that laboratories understand and adhere to CDC recommendations and guidelines. Collaboration and consistent communication with the US Food and Drug Administration and Centers for Medicare and Medicaid Services will be essential in instituting new strategies and addressing HIV testing issues.

References

- 1) Association of State and Territorial Health Officials. (2006) Acute HIV Infection an Opportunity to Enhance Primary Prevention. March.
- 2) Bennett, B. (2007). Chapter 2 HIV testing. HIV/AIDS Primary Care Guide Chapter 2. Retrieved August 10, 2007, from <http://www.faetc.org/Guide/index.asp>.
- 3) Centers for Disease Control and Prevention. (2007) Quality Assurance Guidelines for Testing Using Rapid HIV Antibody Tests Waived Under the Clinical Laboratory Improvement Amendments of 1998. August.
- 4) Centers for Disease Control and Prevention. (1998) Fact Sheet: Human Immunodeficiency Virus Type 2. Retrieved on August 4, 2007 from <http://www.cdc.gov/hiv/resources/factsheets/PDF/hiv2.pdf>
- 5) Joint United Nations Programme on HIV/AIDS (2006) HIV and AIDS Estimates for the United States of America. Retrieved on August 3, 2007, from http://www.unaids.org/en/Regions_Countries/Countries/united_states_of_america.asp
- 6) Morbidity and Mortality Weekly Report. (2001) Revised Guidelines for HIV Counseling, Testing and Referral. November 9 50(19): 1-5
- 7) Morbidity and Mortality Weekly Report. (2004) Notice to Readers: Protocols for Confirmation of Reactive Rapid HIV Tests March 19 53(10):221-222
- 8) PATH-A Catalyst for Global Health (2007) Current Information on Rapid Diagnostic Tests. Retrieved online August 10, 2007 from <http://www.rapid-diagnostics.org/accuracy.htm>

This publication was supported by Cooperative Agreement Number U60/CCU303019 from the Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or imply an endorsement by APHL officers, members, staff or management.