Current Status of Laboratory Testing for HIV in the Philippines

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ABSTRACT

Prevalence of HIV infection in the Philippines remains low. This may be partly due to reliance on passive reporting for surveillance.

The algorithm for laboratory testing for HIV infection has become more stringent in the sense that the screening assays are repeated in the confirmatory centers, before Western Blot is performed. This has been due to the high rate of false positives before 2005.

Nucleic Acid Amplification testing (NAAT) has been performed routinely for blood banking purposes in other countries. In a few pilot studies, it has proven useful in identifying those cases in the early stage of the infection, which are missed on testing by antibody-based assays. The assay may prove useful in knowing whether false negatives happen with the current testing algorithm in the Philippines.

Coupled with the detuned assay, identification of new cases may be critical for prevention of transmission, surveillance of cases, and early medical management if needed.

Key Words: screening, confirmatory, algorithm, enzyme immunoassay, nucleic acid amplification testing, Western Blot

Global and national picture

At the end of 2007, the World Health Organization estimates that there were 33 million people living with HIV. Of these, 2.7 million are thought to have been infected in that year.[1]

The Philippines has one of the lowest rates of HIV infection in Asia. In 2007, WHO estimates the total number of people living with HIV in the country at around 8,000. The same source estimates the total number of deaths due to AIDS from 1990 to 2007 at less than 300.[1]

The WHO recommended assay for screening for HIV is **Enzyme Immunoassay (**EIA), with those samples testing positive being subjected to two more EIA tests. A positive screen is defined as two reactive results out of these three tests. After this positive screen, a confirmatory test is performed, with the use of Western blot.

The strategy of the HIV testing algorithm is to capture all true positives and a few false positives with a highly sensitive screening test and resolve positive specimens with a more specific test for confirmation.

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Evolution of screening assays

Enzyme Immunoassays

Testing for HIV is a two-step process. The first step is the screening test, followed by the confirmatory test. The usual testing algorithm begins with a screening test, with reactive samples being tested again with the same or a different screening test. If that sample is repeatedly reactive (a positive screen), then a Western blot is performed. Screening tests, or assays, include conventional tests (enzyme immunoassays or EIA, and chemiluminescence assays) and rapid tests which have a high sensitivity for serum antibody, and thus give few false-positive results. Confirmatory assays include Western Blots (WB), indirect immunofluorescent antibody assays (IFA), and RNA detection by NAAT (nucleic acid amplification tests). The latter groups of tests have a high specificity.

Since antibody-based tests became available in 1985, screening for HIV infection has gone a long way.

Table 1 shows the generations of EIA assays that have been used for screening over the years. All of them use serum or plasma as test samples. Each class represents a major difference in the principles of the assay, especially the target molecules in the patient's sample.

Four generations of EIAs have been produced since 1985, with latter generations having improved test performance, and have shortened window periods during which antibodies cannot be detected. First and second generation tests have a window period of about 6 to 12 weeks for most individuals.[2] Both generations detect IgG antibodies to HIV. The first generation used viral lysate as a target antigen, while the second generation uses recombinant proteins representing HIV capsid and envelope. As of 2008, second-generation EIAs are the most frequently used HIV screening tests in the USA. [2,3]

Third generation tests have the ability to detect IgM antibodies in addition to IgG, through the antigen sandwich method, in which HIV antibodies from the specimen are sandwiched between two antigen molecules, one in the solid phase and one conjugated to an enzyme such as horseradish peroxidase or alkaline phosphatase. In addition, third-generation tests have the capacity to detect certain HIV subtypes, particularly HIV-1 Group O and HIV-2 which were not included in the previous generation tests.

Fourth generation tests detect IgM and IgG antibodies as well as the presence of the viral capsid antigen p24. The detection of p24 antigen reduces the window period to two weeks, and makes detection of acute HIV infection (before seroconversion) possible. Fourth generation tests combine

Table 1. Enzyme immunoassays for screening for HIV infection.

Generation	Mechanism	Window period	Example
1 and 2	Viral lysate used to bind patient HIV Ab. Detects IgG antibody to HIV viral proteins. Second generation assays are the same as first generation, but use purified Ag or recombinant virus.	4 to 12 weeks	Vironostica HIV-1 Microelisa (bioMerieux, Inc) Genetic Systems rLAV EIA HIV 1 (Bio-Rad Laboratories)
3	Same mechanism as first and second generation, but adds IgM detection, which decreases its window period	3 to 4 weeks	HIVAB HIV1/HIV2 rDNA (Abbott Laboratories) Genetic Systems HIV1/HIV2 plus O EIA (Bio-Rad Laboratories)
4	Same mechanism as third generation, but in addition uses an antibody to detect p24 antigen in the patient's serum	2 weeks	Vidas HIV Duo Ultra (bioMerieux, Inc.)

two methodologies into one assay - antigen and antibody detection. As of 2008, the US FDA has not yet approved any fourth-generation enzyme immunoassay.[4]

NAAT (nucleic acid amplification testing)

The gold standard however for diagnosing acute HIV infection is the use of NAAT in the setting of a negative HIV antibody result. NAAT can be both quantitative and qualitative. Quantitative assays determine the plasma viral load and are used to monitor disease progression and response to antiretroviral therapy. On the other hand, qualitative assays reveal whether HIV RNA is present or not, and are used to screen specimens for the presence of HIV antigen.

Historically, NAAT has not been included in routine HIV-screening protocols, due to the high cost and time and labor that most of those technologies require. To decrease the costs, blood donor programs in the United States have been using pooling algorithms with NAAT [5]. In those algorithms, antibody-negative specimens are combined in pools, and each aggregated screening pool is assessed by NAAT. A negative pool ends the screening protocol. If a pool has a positive NAAT result, then the pool is deconstructed further into either smaller intermediate pools or individual specimens until the positive specimen is identified. The primary benefit of specimen pooling is significantly decreased costs compared to testing each individual specimen. The major drawback is that pooling involves the dilution of specimens, which may impact test sensitivity. In the USA, recent public-health efforts to diagnose acute infection have led to the use of pooling with NAAT in routine HIV screening in certain settings. [6-8]

While third- and fourth-generation EIAs are likely to provide an alternative for acute HIV detection in low income settings, algorithms combining standard EIAs followed by pooled-specimen screening by a NAAT are recommended for settings in which resources are available.

Rapid HIV antibody tests

There are four rapid tests cleared for HIV-1/2 detection, one of which, the OraQuick Advance Rapid HIV-1/2 Antibody Test, is cleared for testing of oral fluid . The sensitivity of the tests is comparable to standard second-generation EIA testing [9]. Rapid tests each have a synthetic antigen (the gp41 region of HIV-1, and gp36 for HIV-2) affixed to a test membrane, and a sample (finger-stick blood, venipuncture blood, or oral fluid) is applied to the membrane. If the sample contains antibodies to the gp41 region of HIV, then the membrane will change color. In addition, each test has a goat anti-human IgG antibody for control, and each test requires the periodic use of external controls.

Each rapid test has an assigned Clinical Laboratory Improvement Amendments of 1998 (CLIA'98) category that determines the personnel and the type of facilities required to perform the test. Persons without formal laboratory training and outside the traditional laboratory can perform waived tests. To classify as a CLIA-waived test, the test must use direct, unprocessed specimens (such as oral or whole blood), and must be easy to perform by persons without formal laboratory training. The rapid test is the only HIV-testing modality that can be done outside of the laboratory setting. Another benefit of the rapid test is that it can be non-invasive, as oral-fluid rapid testing was FDA cleared in 2004. Post-marketing surveillance of the Ora Quick Advance Rapid HIV1/2 Antibody Test on whole blood and oral fluid yielded favorable results.[10]

Urine tests

Urine HIV tests measure intact HIV IgG antibodies found in urine specimens and have been cleared by the FDA for use with EIA and Western Blot. Assays using urine have the potential to reduce barriers to testing, as they are simple and non-invasive,and the urine can be stored for long periods at room temperature. Despite its advantages, the urine-based HIV test is not commonly used, although test performance may be similar to blood-based testing (in one study the Maxim Urine HIV-1 EIA had a sensitivity of 98.7%).[11]

Rapid urine tests, although commercially available, are not FDA cleared. The Aware-Urine assay (Calypte Biomedical Corp., Rockville, MD), initially had promising preliminary results, but the sensitivity was found to be poor in a recent study set in rural Uganda. The study reported that 942 urine samples yielded a sensitivity of 88.7% and specificity of 99.9% in comparison to EIAs using serum confirmed by Western Blot. [11]

Confirmatory Testing

All positive screening tests must undergo a confirmatory test, either a WB or indirect immunofluorescent antibody assay, and most recently the APTIMA HIV 1 RNA Qualitative Assay which was the first nucleic acid amplification test to be cleared by the FDA for confirmatory testing. Confirmatory tests are highly specific, more time consuming, and more expensive than most screening assays. IFA is used less frequently as it is expensive and requires highly trained laboratory personnel.

In WB assays, individual HIV proteins are fractionated by weight via gel electrophoresis and transferred onto nitrocellulose paper. The patient's serum is added to the paper; and if antibodies are present, they will bind to the corresponding antigens. CDC guidelines define a positive result as reactivity to at least two of the major antigens (p24, gp41, and gp120/160), and a negative result requires the absence of all bands. The WB is the traditional confirmatory test, and modified WBs can identify and differentiate between HIV-1 and HIV-2 infections. A reactivity profile that does not meet criteria for either positive or negative results is considered indeterminate.

In contrast to ELISAs or rapid tests that provide results reflecting the reactivities of antibodies to any or all of the antigens indiscriminately for screening purposes, Western blotting (or line immunoassays with separated viral proteins immobilized on membrane testing strips) generate specific information on the reactivities of antibodies toindividual proteins.[12]

The Department of Health testing algorithm

Screening

Republic Act 8504 , Administrative Order No. 2005-0027 states that only licensed or accredited clinical laboratories and/or blood centers are allowed to operate an HIV testing center and shall have a licensed medical technologist with training on HIV proficiency testing.

Testing centers should use only HIV test kits evaluated and recommended by NRL-SACCL-SLH SACCL (STD AIDS Cooperative Central Laboratory of San Lazaro Hospital, Department of Health) , which have been registered by BFAD (Bureau of Food and Drugs) with a valid Certificate of Product Registration (CPR). BFAD shall issue a list of registered kits to the BHFS (Bureau of Health Facilities and Services) for dissemination. BHFS shall monitor testing laboratories on the use of unregistered kits. Violations by the testing center will be given sanctions by the BHFS, while violations by the manufacturer/distributor will be sanctioned by the BFAD.

Prior to testing, all patients must undergo pretest counseling, fill the personal information sheet, and sign an informed consent form. Testing laboratories should follow the algorithm recommended by the NRL (National Reference Laboratory, in this case the SACCL or RITM, the Research Institute for Tropical Medicine). Reactive blood units shall be referred to the NRL for confirmatory testing.

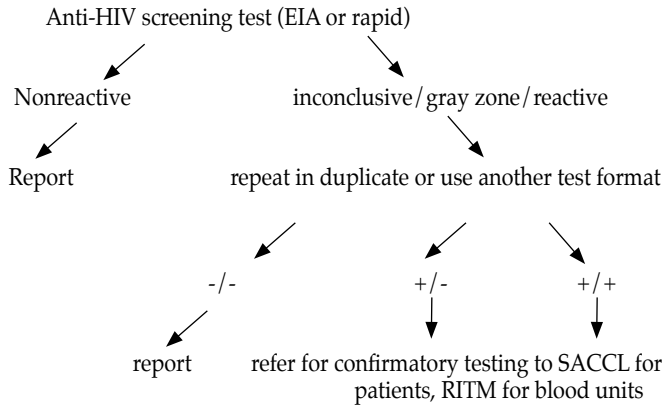

Figure 1. Testing algorithm for Screening Laboratories.

Confirmatory testing

All specimens that test reactive on screening assays anywhere in the Philippines are sent to SACCL or RITM. Before 2005, all specimens that were received were tested by Western Blot. However, because of the high prevalence of false positives, a new algorithm for testing was implemented in 2005. All specimens that were received for confirmatory testing were screened twice, once using rapid testing, and once using a fourth generation EIA. Currently, Serodia HIV1/2 is being used for rapid testing, and a fourth generation HIV1/2 plus p24 are being utilized. A reactive result for any or both of these tests leads to testing by Western Blot.[13]

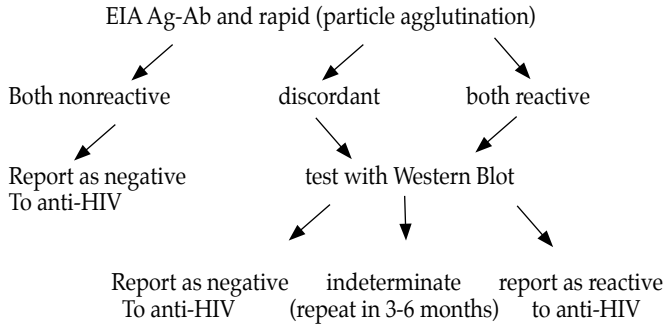

Figure 2. Testing algorithm for Confirmatory Samples.

Table 2 summarizes the results of Western Blot testing that was performed by SACCL since 2005. [13]

The data above shows that consistently, the number of nonreactive specimens is greater than that of reactive specimens, with indeterminate specimens making up less than 5 % of the total number.

A 2007 review by Guan summarized the frequency, causes, and new challenges posed by indeterminate results of the Western blot. In that report, the reported frequency of

Table 2. Western Blot Testing by SACCL, 2005-2007.

Year	Number of Tests Performed	Number of Reactive Specimens	Number of Nonreactive Specimens	Number of Specimens with Indeterminate Results
2005	650	211 (32.46 %)	413 (63.54 %)	26 (4.00 %)
2006	758	289 (38.13 %)	440 (58.05 %)	29 (3.86 %)
2007	809	352 (43.51 %)	431 (53.28 %)	26 (3.21 %)
Total	2217	852 (38.43 %)	1284 (57.92 %)	81 (3.65 %)

indeterminate results from a number of testing sites varied from a low of 2.1 % to a high of 45 %. [14] The above data from SACCL shows that results of indeterminate are comparable elsewhere, although slightly on the lower end of the range.

Potentially useful assays

Figures for HIV estimates in the Philippines are based on passive reporting. As such, estimates of the prevalence of HIV infection in the country can be much higher than the actual number of reactive specimens that were tested by SACCL.

This gap in knowledge can have several implications. One of these is that the lay public may become complacent into thinking that because of the low reported prevalence of HIV infection in the country, there is little risk with unprotected sexual activity or intravenous drug use. The low reported prevalence may also encourage the promotion of "sex tours" that have become popular in other Southeast Asian Countries, with individuals of high risk for HIV infection participating.

In this country we also have little or no data on the approximate points in the infection process when clients get to know of their HIV status**.** Many individuals probably know they are infected when they become symptomatic due to opportunistic infections or tumors. The persistence of the HIV pandemic is due in part to the failure to identify or inform infected individuals. Early diagnosis is important not only because of its benefits to the health of the individual, but also because it has a big role in limiting further HIV transmission.

Experience with testing in other countries has shown several promising assays that help gain more information about the character of the epidemic within their national borders. Such assays or slight modifications of the algorithm can be adapted here, to learn more about the status of the epidemic in this country.

Nucleic acid amplification tests (NAAT)

Among 37,164,054 seronegative blood units screened by the American Red Cross from 1999 to 2002, 12 were confirmed to be positive for HIV-1 RNA — or 1 in 3.1 million donations — only 2 of which were detected by HIV-1 p24 antigen testing.[15]

This data indicate that the implementation of minipool nucleic acid screening likely prevented about 5 cases of transfusion-transmitted HIV-1 infection annually. Despite the fact that the rate is relatively low and has remained stable for five years, implementation of these tests was consistent with the goal of maximizing blood safety. It has been estimated that nucleic acid screening has reduced the residual risk of transfusion-associated infection for HIV-1 to about 1 in 2 million blood units from repeated donors. This is a reduction from **a** rate of 1 in 1.5 million for HIV-1 with the use of serologic testing alone.[15]

In the above group of HIV-positive prospective donors, the assay was able to detect the infection in the early stage of the infection, before they had seroconverted. This information can provide insights into risk factors associated with viral infection and potentially contribute to studies of the natural history, pathogenesis, and treatment of these infections.

Admittedly, this type of testing for HIV in the Philippines might be too expensive for routine use, and would make the cost of blood transfusion screening much higher than what the current prices are. Still, when taken literally, "maximal blood safety" means utilizing the NAAT assay, not only for HIV but also for hepatitis C as well. Therefore, the cost of nucleic acid amplification testing would need to decrease substantially to bring it in line with that of most other accepted medical practices.

NAAT with the detuned assay

Antibodies that appear during the early stages of HIV infection are typically associated with a low titer. Janssen et al.[16] developed a detuned antibody test (the "less sensitive" HIV antibody enzyme immunoassay) that continues to yield a negative result for 170 days, on average, after a standard ("sensitive") antibody enzyme immunoassay test becomes positive[17], thereby allowing the identification of recent infections.

This assay was used, along with NAAT in a study involving 109,250 persons at risk for HIV infection who had consented to HIV testing presented at state-funded sites in North Carolina between November 1, 2002, and October 31, 2003. There were 606 HIV-positive results. Established infection, as identified by standard enzyme immunoassay and Western blot analysis, appeared in 583 participants; of these, 107 were identified, with the use of sensitive–less-sensitive enzyme immunoassay tests, as recent infections. A total of 23 acutely infected persons were identified only with the use of the nucleic acid amplification algorithm. With all detectable infections taken into account, the sensitivity of standard antibody testing was 0.962 (95 percent confidence interval, 0.944 to 0.976). In comparison, the specificity and positive predictive value of the algorithm that included nucleic acid amplification testing were greater than 0.999 (95 percent confidence interval, 0.999 to >0.999) and 0.997 (95 percent confidence interval, 0.988 to >0.999), respectively. Of the 23 acute HIV infections, 16 were detected at sexually transmitted disease clinics. Emergency measures for HIV prevention protected 48 sex partners and one fetus from high-risk exposure to HIV.[18]

This type of algorithm allows for better identification of early infection. There are three principal reasons why the detection of such infections is especially important. First, the recognition of acute HIV infection allows appropriate clinical management. For the infected person, a prompt diagnosis can prevent the administration of inappropriate tests and therapies often used to evaluate and treat the symptoms of acute retroviral infection. When indicated, antiretroviral therapy can be provided. The hypothesis that even short-term antiretroviral therapy that is initiated in patients with acute HIV infection may delay the progression of disease is currently being evaluated in clinical trials.[18-20]

Second, the identification of persons with acute HIV infection can help prevent further transmission of the virus. The probability of transmission is high during the first few months after acute HIV infection[21], during which time patients have a high viral burden in the blood and genital tract and are likely to engage in risky sexual behavior[21]. Standard voluntary counseling and testing practices for HIV identify many patients as HIV-positive with advanced disease[22,24], after most sexual transmission is likely to have occurred already. This study demonstrated that notification of results, access to antiretroviral therapy, and partner counseling and referral services can be instituted for acutely infected persons within days after testing. Third, the identification of acute infections can improve HIV surveillance. Standard antibody tests limit surveillance to the monitoring of populations living with latent or advanced HIV disease.[18]

Conclusions

The country has consistently reported a low prevalence of HIV infection, and part of the reason for this is the reliance on passive reporting. While this is acceptable and perhaps is being used in most countries worldwide, it underestimates the prevalence of infection.

In the confirmatory portion of the testing algorithm, the initial steps repeat the screening procedures. This was done mainly because of the high rate of false positives when all specimens are subjected to the Western blot procedure directly at the National Reference Laboratories. While this is reasonable as Western blot is expensive, time consuming, and technically demanding, the additional step has not been validated extensively with the NAAT to be too restrictive as to produce false negatives, which are conceivable when there is early infection and seroconversion has not yet taken place. Experience in other countries have shown that NAAT is very useful is diagnosing early infection.

The detuned assay can be made use of, especially in tandem with the NAAT, to know when cases are in the early stage of the infection. In this scenario, surveillance with possible contact tracing and counseling can be more focused to prevent behaviors that lead to transmission, as it is during this phase that transmissibility is greatest. Medical management can also be effected early, although it may still be controversial at this point.

The number of cases of HIV infection, as reported after Western Blot confirmation remains low in comparison with several countries near the Philippines. This may be partly due to reasons already cited above. With the use of other assays it may be possible to discern a more accurate picture of what is happening, and hopefully keep the low prevalence from becoming high.

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