SHORT COMMUNICATION

Evaluation of Three Commercial Rapid Tests for Detecting Antibodies to Human Immunodeficiency Virus

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Summary

Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest are simple/rapid tests for the detection of antibodies to HIV-1 and HIV-2 in human whole blood, serum and plasma samples. The assay is one step and the result is read visually within 15 minutes. Using 92 known HIV-1 reactive sera and 108 known HIV-1 negative sera, the 3 HIV tests correctly identified all the known HIV-1 reactive and negative samples. The results indicated that Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV are as sensitive and specific (100% concordance) as Microparticle Enzyme Immunoassay. The data indicated that these 3 HIV tests are effective testing systems for diagnosis of HIV infection in a situation when the conventional Enzyme Immunoassay is not suitable.

Key Words: Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK, PenTest, MEIA, Sensitivity and specificity, Human immunodeficiency virus

Introduction

The World Health Organization reported that by June 2000, there were 34.3 million adults and children living with infected HIV globally[1]. In 1999, there were 5.4 million people newly infected with HIV and the total global number of AIDS deaths since the beginning of the epidemic was 18.8 million. More than 90% of . people with HIV/AIDS were reported from the developing countries, in which 24.5 million of HIV/AIDS cases were reported in sub-Saharan Africa and 5.6 million in South & South-East Asia. The outlook for HIV epidemic in Asia and Pacific region is grave. China, with a fifth of the world's population registered a rise of 67% in reported HIV infections in the first six months of 2001. Together with India, the 2 countries account for more than one-third of the world's population and even a comparatively low HIV prevalence rates can be translated into a large numbers of infections in the region. In Malaysia, there were 49000 adults and children living with HIV/AIDS at the end of 1999. The estimated numbers of AIDS death were 1900 and 0.42% of the adults 15-49 year old in the country were living with HIV/AIDS.

Laboratory diagnostic support is an important integrated part of health care in the fight against the HIV/AIDS epidemic. Reliable diagnostic tests are instrumental in surveillance surveys by collecting epidemiological data to monitor the

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spread of human immunodeficiency virus. Globally, especially from the developing countries, there has been request for access to cheaper and effective antiretroviral drugs to help in the battle against AIDS. However, it should be remembered that before treating and caring for patients, the infection must be diagnosed correctly. Until today, voluntary counseling and HIV testing are the most effective methods in the fight against the HIV/AIDS epidemic. The use of reliable HIV tests and appropriate testing strategies is important in the prevention of mother-to-child transmission. Another preventive measure in most countries is the use of mass media like newspaper, television and radio, organized activities and educational programs in schools and in the community to educate the public on HIV disease. Anonymous HIV testing centers have been established in some STD clinics, family planning clinics and hospitals to encourage HIV screening among high-risk individuals. HIV counseling service or hot line has been organized by various government agencies and non-governmental organizations (NGO) to provide information and offer advice to the public seeking more information on HIV infection.

HIV antibody test was first introduced in 1984 and since then, there has been a rapid evolution in HIV diagnostic technology. Today, a wide range of HIV antibody test of different format is available in the market. Enzyme-linked immnuosorbent assays (ELISAs) are most widely used and they are usually the first screening assays performed on potentially HIV-infected patients. These tests have high sensitivity and specificity and are able to detect both HIV 1/HIV-2 and HIV variants. ELISAs are designed specifically for screening large numbers of specimens making them particularly suitable for use in surveillance and large centralized blood bank. As ELISAs require sophisticated equipment and experienced laboratory technologists to perform the tests, the setup and their use is limited to major hospitals with high laboratory volume loads.

Although ELISA testing is now available in most hospitals in the country, the delivery of such service to the public is hindered by many problems. In an out-patient clinic, the standard HIV testing procedure using conventional ELISA may take up to 2 weeks and requires the patient to return to the out-patient clinic at least twice. Further delay and inconvenience will occur if the patient has to make an extra trip to the clinic for repeat testing if the first sample was found to be HIV antibody positive. The inconvenience and the time to get the test results discouraged many patients to come forward for HIV testing or return for test results or post-test counseling[2].

On-site, simple/rapid HIV tests are now available commercially using agglutination, comb or capillary and dipstick formats. These tests are based on agglutination, immuno-dot, immuno-chromatographic or immuno-filtration technology. These simple/rapid assays require little or no additional equipment and are designed for use in laboratory with a limited number of samples. These tests are easy to perform, require no experienced laboratory technologist and the results are available within 10 to 15 minutes, thus making them more economical than ELISAs in low throughput laboratories. The majority of simple/rapid HIV tests can be stored at room temperature for extended period of time. The tests are most suited for use in HIV voluntary counseling and testing centers and antenatal clinics where making same day testing and results available in a single visit can result in timely treatment interventions.

The objective of this study is to determine the sensitivity and specificity of three simple/rapid HIV tests by comparing with the conventional MEIA HIV-1/2 (Abbott Laboratories, USA). The 3 simple/rapid HIV tests are PenTest HIV, a capillary flow assay, Determine HIV-1/2 and Chembio HIV 1/2 STAT-PAK, both immunochromatographic rapid assays for the detection of antibodies to HIV-1 and HIV-2 in human whole blood, serum and plasma.

Materials and Methods

Blood samples

The evaluation was conducted between August 2002 to October 2002. To study the sensitivity and specificity of Determine HIV-1/2, PenTest HIV and Chembio HIV-1/2 STAT-PAK, a total of 200 frozen sera were used in which 92 sera were known HIV-1/2 reactive and 108 HIV-1/2 negative. Among the HIV-$^1/_2$ negative sera, 35 sera were HIV-1/2 indeterminate by MEIA and 73 known HIV-1/2 negative samples. The reactive samples were first tested by MEIA (Microparticle Enzyme Immunoassay, Abbott Diagnostic, USA), PA assay (Serodia HIV-1/2, Fujirebio Inc, Japan) and confirmed by Inno-Lia HIV (Innogenetics N.V., Belgium). The 35 sera with indeterminate HIV-1/2 results were all MEIA reactive but negative by both PA assay and Inno-Lia HIV, these samples were subsequently reported as negative to HIV-1/2 antibodies. The 73 HIV-1/2 negative samples were all screened by MEIA and negative for HIV-1/2 antibodies. The samples were obtained from the Blood Bank, Antenatal Clinic and in-patients of University Malaya Medical Centre (UMMC).

Laboratory evaluation of simple/rapid assays. Determine HIV-1/2 is a one step immunochromatographic assay for the qualitative detection of antibodies to HIV-1/2. The assay is based on sandwich immunoassay and uses a nitrocellulose strip with a conjugate site containing HIV-1 and HIV-2 *env* proteins conjugated to selenium colloid and a capture site containing HIV-1 and HIV-2 antigens. A procedural control bar is incorporated in the strip. The reagents for the control bar are immobilized at the control window located at the upper side of the patient window. 50 µl of serum is placed on the sample application pad, followed by the addition of 1 drop of chase buffer. The result is read visually within 15 minutes. The positive result is indicated by 2 bars i.e. a red bar appearing in both the control window and the patient window of the strip. A negative result is indicated by a red bar in the control window and no red bar appearing in the patient window. If there is no red bar in the control window of the strip, the result is invalid and the test should be repeated.

Chembio HIV-1/2 STAT-PAK (Chembio Daignostic Systems, USA) is a rapid immunochromatographic test, the specific antibody binding protein conjugated on colloidal gold dye particles and HIV-1/2 antigens are bound to the membrane solid phase. 5 µl of serum is placed on the SAMPLE (S) using a disposable 5 µl sample loop followed by 3 drops of running buffer. The result is read visually within 10 minutes. A positive result is indicated by two pink/purple colored lines, one line in the TEST (T) area and one in the CONTROL (C). The intensity of the line in the TEST (T) varies with the concentration of HIV antibodies, a barely visible line in the area is considered positive to HIV-$^1/_2$ antibodies. A negative result is indicated by one pink/purple coloured line in the CONTROL (C) with no colored line in the TEST (T) area. A pink/purple colored line must always appear in the CONTROL area for the test to be accepted.

PenTest HIV (Noventis (S) Pte Ltd) is a capillary flow assay. All reagents except buffer are provided on solid phases in a dried format on a test strip inside the transparent cylinder or Pen having a capillary tip. The buffer reagent is in a separately sealed cup. The capillary tip of the Pen is used to collect the serum, a few microliters of serum is taken up by capillary action into the distal tip of the cylinder. The Pen is then inserted into the cup. Immunochromatography is initiated when a mixture of specimen and buffer contacts the test strip. The specimen/buffer mixture migrates along the strip by capillary action and reconstituting a dye conjugate. The result is read visually within 15 minutes. A positive result is indicated by the appearance of 2 lines, a TEST LINE closer to the bottom of the test strip and CONTROL LINE on the top of the strip above the TEST LINE. A visible TEST LINE, regardless of its intensity, is considered as reactive to anti-HIV-1/2 antibodies. A negative result is indicated by non-reactive TEST LINE with a reactive CONTROL LINE. The test is considered invalid if the CONTROL LINE is not reactive.

The three simple/rapid HIV tests were compared to a fully automated MEIA system and PA assay if the sample was found to be initially reactive to anti-HIV-1/2 antibodies. MEIA is an in-vitro assay for the qualitative detection of antibodies to HIV 1 and 2. It has an average throughput of 80, up to 120 tests per hour. The result turnaround time for STAT assay was 15 minutes. The assay procedure recommended by the manufacturer was followed and it took about 45 minutes to complete the assay. The initially reactive samples were retested and only the repeated reactive samples were considered as positive.

PA assay (Serodia HIV-1/2, Fujirebio Inc, Japan) is a passive particle-agglutination test for the detection of antibodies to HIV-1 and 2. The assay was used as a supplementary test to confirm initially reactive sample by MEIA. The results were read visually according to the criteria suggested by the manufacturer. The entire assay procedure took about 2 hours and 30 minutes. Inno-Lia HIV (Innogenetics N.V., Belgium) is a line immunoblot assay with recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O coated as discrete lines on a nylon strip with plastic backing. The test was used to confirm all MEIA initial or repeatedly reactive sera. The test procedure, incubation and validation of results were based on the procedure recommended by the manufacturer.

Statistical analysis

Frequency tables and cross-tabulations were constructed for the use of calculating sensitivity, specificity and positive predictive value (PPV). When comparing the Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV to the MEIA, we assumed MEIA as the gold standard and calculated the sensitivity and specificity relative to MEIA.

Results

In the study, Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV detected all the 92 HIV-1/2 antibodies reactive sera. The 73 known HIV-1/2 negative sera were also reported as negative by all 3 simple/rapid HIV tests (Table I). The results indicated that the performance of Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV were comparable to MEIA for the diagnosis of HIV infection.

The 35 indeterminate sera by MEIA were correctly identified as negative by all the 3 simple/rapid HIV tests. The data showed that Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV were as sensitive and specific (100% concordance) as MEIA (Table II). All the known HIV-1/2 reactive sera were correctly detected by the 3 tests. The 108 HIV-1 negative sera were also reported to be negative by the 3 simple/rapid HIV assays. This resulted in a specificity estimate of 100% and a positive predictive value of 100%, suggesting no HIV negative subjects were falsely tested positive.

Table I: Laboratory Evaluation of Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV

Type of sample*	Determine HIV-1/2		HIV-1/2 STAT-PAK		PenTest HIV	
	pos	neg	pos	neg	pos	neg
HIV reactive (N=92)	92	0	92	0	92	0
HIV indeterminate (N=35)	0	35	0	35	0	35
HIV negative (N=73)	0	73	0	73	0	66**

*HIV reactive: MEIA, PA and Inno-Lia HIV positive
HIV indeterminate: MEIA positive but , PA and Inno-Lia HIV negative
HIV negative: MEIA negative
**Only 193 test pens supplied for evaluation.

		MEIA	
		Positive	Negative
simple/rapid HIV tests *	Positive	92	0
	Negative	0	108

Sensitivity and specificity = 100%

* Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV

Table II: The Sensitivity and Specificity of Simple/Rapid HIV Tests Compared to MEIA

Discussion

Overall, the results of this study indicated that the sensitivity and specificity of Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV are comparable to that of conventional EIA used for the detection of anti-HIV antibodies. The 3 simple/rapid HIV tests successfully detected all the HIV-1/2 reactive and HIV seronegative blood samples, implying a 100% sensitivity and specificity as compared to conventional screening methods. In comparing the performance of rapid Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest HIV assays to the MEIA, the results showed that the rapid assay was as sensitive and specific (100% concordance) to the more complex, technically demanding MEIA.

The sensitivity and specificity of rapid HIV tests are comparable to conventional EIA [3,4]. The HIV rapid tests available in Malaysia include SimpliRED HIV-1/HIV-2 (Agen Biomedical Ltd, Australia), SimpliDRY HIV-1/HIV-2-AB (Simplidry Sdn Bhd, Malaysia), Uni-Gold HIV (Trinity, Ireland) and Hema. Strip HIV-1/2 (Saliva Diagnostic Systems, USA). The sensitivity and specificity of SimpliRED HIV-1/HIV-2 [5], SimpliDRY HIV-1/HIV-2-AB (Ng et al, unpublished data), Hema. Strip HIV-1/2 [6] have been studied and are found to be comparable to conventional EIA method.

It is recognized that HIV screening is an important tool in the fight against the HIV/AIDS epidemic. However, the failure of large numbers of patients returning to the clinic for results and post-test counseling has indicated the need of developing a new and more effective HIV screening strategy. The rapid on-site HIV tests that can provide results and result-specific counseling on the day of initial visit has potential to compliment the weakness of the current laboratory services and increase the efficiency of HIV screening and testing. Farnban et al[2] reported that HIV screening using rapid assay is more cost-effective than the conventional EIA procedure if the test-positive patients are given preliminary screening test results. But the concern of a preliminary false positive results, the cost of rapid tests and the lack of facilities and resources to provide pre-test and result-specific counseling on the same day are limiting the use of rapid HIV tests. In a standard HIV testing protocol, the diagnosis of HIV infection required a screening

test (usually EIA) and a second supplementary testing system that has a different test format to the screening test (usually PA) if the initial screening test was reactive. In practice, rapid test can be used as a screening test and EIA as a supplementary testing system. This will allow the majority of HIV-free patients to obtain their results and pre-test counseling in a single visit; a second blood sample can also be obtained from initially reactive patient in the first visit for retest using EIA. Kassler et al (1977)[7] reported a 210% increase for uninfected patients and a 23% increase for infected patients received their test results; a 109% increase of HIV-positive clients returned for confirmatory results and post-test counseling with the use of on-site rapid HIV testing with same-day results and counseling. Studies also indicated that the majority of patients preferred the rapid test and the counselors accepted the new testing procedures. There was no evidence to indicate of increasing stress among the counselors or patients [2]. There was a saving of US$11 per test using rapid test and counseling associated with using rapid test does not appear to be less effective than standard pre- and post-test counseling [7].

No single HIV screening assay is appropriate for all situations. The use of more sophisticated and technically demanding ELISA is only suitable in major testing centers with trained technicians and high volume loads [8]. The simple/rapid HIV-1/2 assay is easy to performance, requires no sophisticated and expensive equipment and is more suitable for use in a small rural health clinic or laboratory with low specimen loads. All the simple/rapid assays in the current format are stable at room temperature and require no refrigeration, allowing these tests to be used in rural health centers where cold room or refrigerator may be not available.

The evaluation of Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest in the laboratory using frozen sera did not encounter any technical problems. The assay procedure involved only one step and the interpretation of result visually by the presence of single (HIV negative) or double bands (HIV positive) on the nitrocellulose strip. The results of this study demonstrated that Determine HIV-1/2, Chembio HIV-1/2 STAT-PAK and PenTest have a sensitivity and specificity estimate of 100% and a positive predictive value of 100%. We conclude that these 3 simple/rapid HIV-1/2 assays are effective and user-friendly and can be used as a screening HIV test in situation where HIV result and counseling can be given in a single visit.

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