

Local evaluation of a rapid HIV assay for use in developing countries

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Summary

We present a research scheme for evaluating inexpensive HIV rapid tests in a developing country setting and assess the field validity of the Sero-Strip HIV 1/2 rapid test. The research design features the random allocation of 100 true HIV-positive and 100 true HIV-negative serum specimens to 4 groups, followed by blind testing for HIV status. After one short training session, laboratory technicians at 4 township hospitals (25–35 beds) located 20–50 km from Yangon, Myanmar were sent 800 sera labelled with only an identification number and divided into four groups of 200 specimens each, half being HIV-positive and half HIV-negative. Testing was done in the field with the Sero-Strip HIV 1/2. Determination of the test's validity was based on 399 true HIV positive and 401 true HIV negative sera. All true positives were correctly identified, as were all but two of the true negatives. The sensitivity (% of true positives that test positive) was 100%, and the specificity (% of true negatives that test negative) was 99.5%. The research was completed by in-country scientists who are best suited to evaluate the validity of HIV tests conducted in local environments.

keywords HIV antibody testing, rapid tests, sensitivity, specificity, developing countries, research designs, Myanmar

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Introduction

Transmission of the human immunodeficiency virus (HIV) can be avoided if HIV antibody testing finds blood to be contaminated (Foster & Buve 1995), sexual partners to be infected (Hira *et al.* 1997) or pregnant women to be carriers (Connor *et al.* 1994; CDC 1998). Perhaps no more than 5% of those who are infected in developing countries know they carry the virus, a figure that is likely to be much lower in rural regions. Yet in much of the developing world, HIV testing remains expensive and often elusive, due in the public sector to nonsustainable international support for the purchase of test kits and in the private sector to limited competition over price and quality in the marketplace.

Testing has become increasingly common in Thailand, both of blood donations and of pregnant women (Phuapradit *et al.* 1995). While testing of sexual partners is not routinely done in Thailand, the need is becoming more apparent, as investigations report high male-to-female transmission, even among married couples (Nagachinta *et al.*

1997). Myanmar is economically much poorer than Thailand. The occurrence of HIV is similar to Thailand, as estimated by the United Nations AIDS Programme (1998) and by each nation (see Figure 1). Thus the need for HIV testing is also similar in the two countries. Unfortunately, in Myanmar the only national-level testing being offered by the government is for blood transfusions, with many of the test kits coming from UNAIDS and the United Nations Development Programme (UNDP). In the private sector, HIV tests are being sold, but many are not licensed by the government and the quality remains to be assessed.

To increase access to HIV testing in Asia and other developing regions, internal markets need to be created for private sector sales of valid and inexpensive HIV test kits. Yet to ensure that public health interests are served, test kits should be evaluated in local settings before approval is granted by the government. We present such a study in Myanmar of HIV test kits for rural hospitals lacking adequate laboratory facilities.

Methods

Specimens and HIV assays

Serum specimens for the evaluation were collected from the twice-yearly sentinel surveillance programme and assembled by the National Health Laboratory (NHL) in Yangon, Myanmar. The NHL is the central reference laboratory for all diagnostic tests in the country, including HIV. For our study, each specimen was first tested with the Genelavia HIV Mixt enzyme immunoassay (EIA) (Sanofi Diagnostics Pasteur, Inc., Chaslea, MN.) and then confirmed reactive or nonreactive with the Vironostika HIV Mixt EIA (Organon Teknika, B.V., Boxtel, the Netherlands) and the Detect HIV EIA (Biochem Immunosystems, Inc., Allentown, PA.), following UNAIDS Testing Strategy III (Anonymous 1997). For the study, the NHL used 189 HIV-positive specimens and 152 HIV-negative specimens, divided into 399 vials with HIV-positive sera and 401 vials with HIV-negative sera (Table 1). The mean optical density to cutoff value (OD/COV) of the EIA for the first of the three assays (i.e. Genelavia) performed on each specimen was 0.619 for the 401-HIV negative sera (range: 0.376–0.908) and 13.397 for the 399 HIV-positive sera (range: 6.890–20.832). The HIV test to be evaluated was the Sero-Strip HIV 1/2 (Saliva Diagnostic Systems, Inc., Vancouver, WA). This strip test is done in three steps: first, approximately 200 μ l of buffer is dispensed into a test tube; second, approximately 1 μ l of serum is added with a specimen transfer loop; and third, a test strip is placed into the test tube. If the specimen is reactive, two lines appear within several minutes in the middle of the test strip. If the specimen is nonreactive, only one line appears. If there is no serum or if the serum volume is inadequate, no line appears.

Study design and field procedures

As shown in Figure 2, our intent for the evaluation was to have the NHL prepare 400 HIV-positive and 400 HIV-negative

vials. Unfortunately, one specimen was inadvertently mis-coded so in actuality there were 399-HIV positive vials and 401 HIV-negative vials. The NHL divided the 800 specimens into eight groups of either 100 positive or 100 negative sera (Figure 2). Sera were coded only with identification numbers, with no indication of positive or negative HIV status. Thereafter specimens were assembled in four groups, each with 100 positive and 100 negative sera, but blindly coded, for assessment by field staff. An original vial for each specimen sent to the field was maintained at the NHL.

The field evaluation was conducted at 4 township hospitals (25–35 beds) located 20–50 km from Yangon. Four laboratory technicians from the respective hospitals were brought to Yangon and trained one morning by the National Health Laboratory (NHL) staff on intent of the study, use of the tests and recording of results. Instruction booklets were prepared in the Myanmar language. As part of the training, each technician conducted 10 tests with the Sero-Strip HIV 1/2 and interpreted the findings (one strip if nonreactive, two strips if reactive). The blinded specimens were taken to each township hospital, where the laboratory technicians made the measurement and recorded their results. The kits were used in settings where the daily temperature varied from 14.4 to 32.6 °C (58–91 °F) and the average humidity was 68%. Although electricity was available in each setting, the kits did not require continuous refrigeration. The results were decoded and independently analysed by two of us (MZ and RRF) in our respective countries.

Results

The validity of the Sero-Strip HIV 1/2-test was determined using 800 specimens. Due to an initial coding error, one true positive was actually a true negative. Thus 399 true positives and 401 true negatives were sent to the 4 field sites. As seen in Table 2, all true positives were correctly identified by the township hospital laboratory technicians, as were all but two of the true negatives. Based on these results, the sensitivity

Table 1 Original serum specimens prepared by the National Health Laboratory

True HIV positives (<i>n</i> = 399)			True HIV Negatives (<i>n</i> = 401)		
Identical specimens	Frequency	Number in study	Identical specimens	Frequency	Number in study
1	7	7	1	2	2
2	165	330	2	76	152
3	6	18	3	56	168
4	11	44	4	11	44
			5	7	35
Total	189	399	Total	152	401

Table 2 Validity in field of Sero-Strip HIV 1/2

	True HIV antibody status	
	+ve	-ve
Hospital laboratory technician assessment using Sero Strip HIV 1/2		
+ve	399	2
-ve	0	399
Total	399	401

Sensitivity 100.0%; Specificity 99.5%

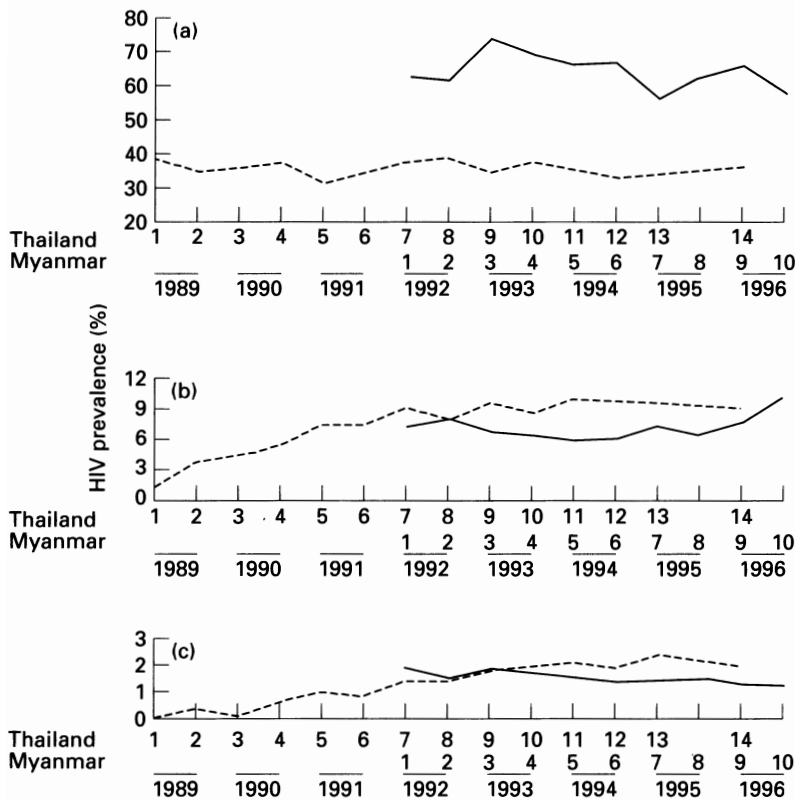


Figure 1 Prevalence of HIV among (a) intravenous drug users, (b) men attending STD clinics and (c) women attending antenatal clinics, sentinel surveillance systems. - - - Thailand (1989-96); — Myanmar (1992-96).

(% of true positives that test positive) of the Sero-Strip HIV 1/2 was 399/399 or 100%; the specificity (% of true negatives that test negative) was 399/401 or 99.5%.

After the study was completed, the two false positive specimens were reanalysed for clerical errors (there were none) and retested by the NHL with the Sero-Strip HIV 1/2 using the remainder of the field specimen and the paired original specimen held at the NHL. The paired original specimen was also tested by the NHL with the Genelavia HIV Mixt EIA and the Immunocomb II HIV 1 & 2 BiSpot (Organics, Ltd). The findings for the two false positives are presented in Table 3, and suggest that contamination of the two specimens in the field or laboratory may have caused the discrepancy.

Discussion

The research was completed by in-country scientists who are best suited to evaluate the validity of HIV tests in rural settings where adequate laboratory facilities are often lacking. We recommend that such applied research become part of the national licensing procedures in developing countries, similar to what now exists in wealthy societies. In addition,

the local equivalent of the Food and Drug Administration should obtain a licensing fee from the manufacturer so that the quality of test kits can be monitored by local researchers over time in the often harsh physical environment.

Others have suggested that HIV tests should only be considered for local marketing after first being approved by the regulatory body of the country of production (Schopper & Vercauteren 1996). Such a restriction would place a considerable cost burden on the manufacturer of potentially inexpensive tests most important to developing countries, causing prices to be set at unacceptable levels. For example, until recently there has not been much interest in the United States in rapid tests and thus few expensive large-scale evaluation trials necessary for Food and Drug Administration approval (Kassler *et al.* 1995) have been done. Yet in less developed international settings, several authors have stressed the urgent need for rapid tests requiring minutes or hours and a single visit vs. two visits over several weeks required with regular testing (Mashu *et al.* 1997; McKenna *et al.* 1997; Ray *et al.* 1997; Stetler *et al.* 1997). To address such demand for simple nonlaboratory tests, governments in the developing world will need to encourage manufactures to produce low-cost HIV tests not

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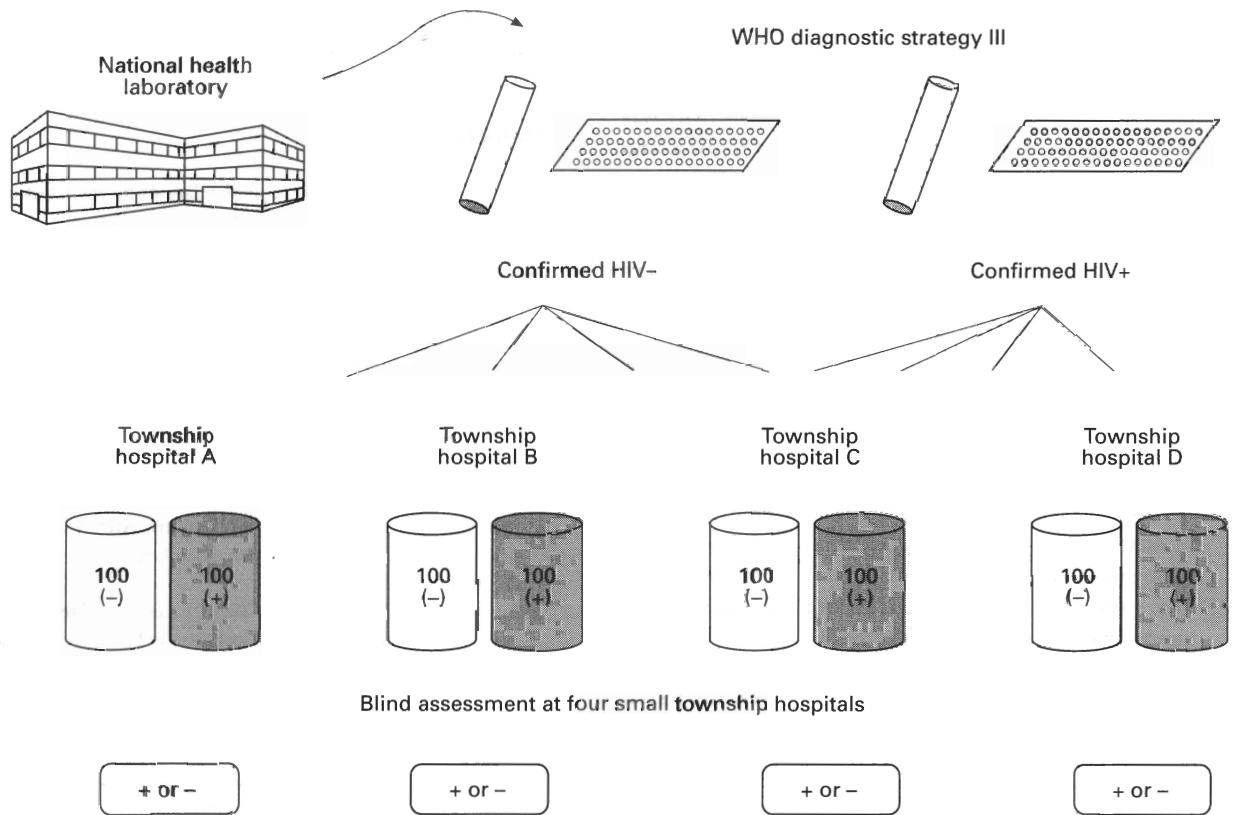


Figure 2 Study design for field evaluation of HIV testing.

intended for developed country markets, but be aware that the accuracy of the test must be locally evaluated, as must quality control over time.

In our study, the Sero-Strip HIV 1/2-test performed very

well in the hands of local technicians at 4 small township hospitals near Yangon. In Thailand, the same test was evaluated in a small urban laboratory. Based on 141 true positive and 448 true negative specimens, both sensitivity

Table 3 Confirmation of two false positive specimens

False positive specimen HIV Assay	One	Two
Sero-strip HIV 1/2-test in field		
Field serum by laboratory technician (blind)	(+)	(+)
Identical field serum by laboratory technicians at other field sites (blind)	(-) (-)	(-) (-) (-)
Sero-strip HIV 1/2-test in NHL		
Duplicate analysis of field serum by NHL staff (not blind)	(+*), (-)	(+) (+)
Duplicate analysis of paired original serum by NHL staff (not blind)	(-) (-)	(+) (+)
Other tests in NHL		
Genelavia HIV Mixt of paired original serum (not blind)	(-)	(-)
Immunocomb II HIV 1 & 2 BiSpot of paired original serum (not blind)	(-)	(-)
Genelavia HIV Mixt reanalysis of original serum for identical specimens (not blind)	(-) (-) (-)	(-) (-) (-) (-)
Vironostika HIV Mixt reanalysis of original serum for identical specimens (not blind)	(-) (-) (-)	(-) (-) (-) (-)
Detect HIV reanalysis of original serum for identical specimens (not blind)	(-) (-) (-)	(-) (-) (-) (-)

(+) positive HIV reaction; (-) negative HIV reaction; * weak positive reaction

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and specificity were reported as 100% (Pagcharoenpol *et al.* 1996). At a large urban hospital in Sao Paulo, Brazil, 109 HIV-positive and 680 HIV-negative specimens were blindly tested with the Sero·Strip HIV 1/2-test; the sensitivity was 99.1%, the specificity, 99.9% (Eskes *et al.* 1996). At a large urban hospital in Mexico City, Mexico with 1541 HIV-positive and 1387 HIV-negative specimens, the sensitivity and specificity of the Sero·Strip HIV 1/2 assay was measured as 99.7% for both (Burgess-Cassler *et al.* 1996). Finally, the test has been evaluated by UNAIDS, found to conform to their minimum requirements for sensitivity and specificity, and is included in their bulk purchase program. Thus our findings using local laboratory workers are similar to those of other researchers in multiple international settings.

Recently investigators in several Asian countries have reported that infected husbands are the main cause of HIV infection among unsuspecting wives, thereby stimulating interest in testing and partner notification programmes (Gangakhedkar *et al.* 1997; Nagachinta *et al.* 1997). Others have stressed the importance of HIV testing and short dose zidovudine treatment to prevent mother-to-child transmission (CDC 1998). Yet cost remains a formidable barrier to detection efforts, unless new inexpensive tests and testing strategies can be developed. UNAIDS has kept cost in mind by recommending that a single assay be used for testing blood transfusions, or when conducting surveillance if the prevalence of HIV is greater than 10% (Anonymous 1997). For diagnostic purposes, however, they recommend that at least two tests be used if the prevalence is believed to be above 10%, or three tests when the prevalence is thought to be below 10%. Stetler and associates have shown that two tests (i.e. UNAIDS Strategy II) worked well in rural Honduras, even in low-prevalence populations (Stetler *et al.* 1997), while Wilkinson *et al.* (1997) have suggested, based on their findings in South Africa, that one rapid test may be sufficient, especially in resource-poor settings. We concur with Wilkinson *et al.* (1997) that multiple testing strategies should be questioned and evaluated, taking into account cost and feasibility, and the consequences of not testing those who carry the virus. All such strategies, however, need access to simple, accurate, inexpensive tests, which need to be evaluated in local settings where laboratory facilities are often lacking.

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was formerly employed by Saliva Diagnostic Systems, and still owns stock in the company.

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