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

Myint Zaw¹, Ralph R. Frenrichs¹, Khin Yi Oo¹ and Nora Eskes

1 AIDS Prevention and Control Program, Yangon, Myanmar
2 Department of Epidemiology, UCLA, Los Angeles, USA
3 National Health Laboratory, Yangon, Myanmar

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

correspondence Professor R. R. Frenrichs, Department of Epidemiology, UCLA, Los Angeles, CA 90095–1772, USA

Introduction
Transmission of the human immunodeficiency virus (HIV) can be avoided if HIV antibody testing finds blood to be contaminated (Foster & Buse 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 to 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 (Phuaaprit 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, international market needs 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 \( \Delta \) diagnostic tests in the country, including HIV. For our study each specimen was first tested with the Genelisa HIV MST enzyme immunoassay (FIA) (Sanofi Diagnostics Pasteur, Inc., Chaldea, MN, USA) and then confirmed reactive or nonreactive with the Vironostika HIV-MST EIA (BioMerieux Teknika, B.V., Bostel, the Netherlands) and the Denvax HIV EIA (Bachem Immunoassays, Inc., Allenstown, PA), following UNAIDS Testing Strategy III (Anonymous 1997). For the study the NHL used 189 HIV-positive specimens and 352 HIV-negative specimens, divided into 399 viials with HIV-positive sera and 401 viials 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. Genelisa) performed on each specimen was 0.619 for the 401 HIV-negative sera (range: 0.376–0.900) and 0.397 for the 399 HIV-negative sera (range: 0.890–20.832). The HIV test to be evaluated was the SeroStrip HIV 1/2 (Saliva Diagnostic Systems, Inc., Vancouver, WA): This strip test is done in three steps: first, approximately 200 \( \mu \)l of buffer is dispensed into a test tube; second, approximately 1 \( \mu \)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 oat 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 FSV-positive and 400 FSV-negative

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Original serum specimens prepared by the National Health Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>True HIV positives (n = 399)</td>
<td>True HIV Negatives (n = 401)</td>
</tr>
<tr>
<td><strong>Identical specimens</strong></td>
<td><strong>Number of specimens in study</strong></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>165</td>
<td>330</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>189</td>
</tr>
</tbody>
</table>

Unfortunately, one specimen was inadvertently mis-coded so in actuality there were 399 HIV-positive viials and 401 HIV-negative viials. 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 viial 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 SeroStrip HIV 1/2 and interpreted the findings (one strip if reactive, 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°C 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 analyzed by two of us (MZ and RKF) in our respective countries.

Results

The validity of the SeroStrip 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>
<thead>
<tr>
<th>Table 2</th>
<th>Validity in field of SeroStrip HIV 1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True HIV antibody status</strong></td>
<td><strong>Hospital laboratory technician assessment using SeroStrip HIV 1/2</strong></td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td><strong>Number of specimens</strong></td>
</tr>
<tr>
<td>+ve</td>
<td>399</td>
</tr>
<tr>
<td>–ve</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>399</td>
</tr>
</tbody>
</table>

Sensitivity 100.0%, Specificity 99.5%.
Figure 1 Prevalence of HIV among (a) injecting drug users, (b) men attending STD clinics and (c) women attending antenatal clinics. Sentinel surveillance 1989-1995. *Myanmar 1992-96.

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 (Scheper & Vercauteren 1996). Such a restriction would place a considerable cost burden on the manufacturer of potentially expensive 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. Need for Food and Drug Administration approval (Kasule et al. 1992) has 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; Smart et al. 1997). To address such demand for simple nonlaboratory tests, governments in the developing world will need to encourage manufacturers to produce low-cost HIV tests not
null
and specificity were reported as 100% (Paghchaoen 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 Serostrip HIV 1/2 test; the sensitivity was 99.1% and specificity, 99.9% (Takes 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 Serostrip HIV 1/2 assay was measured as 100% for both (Burgos-Cordero 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 programs (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 testing 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 0.5% (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%. Stettler and associates have shown that two tests (i.e. UNAIDS Serology II) worked well in rural Honduras, even in low-prevalence populations (Stettler 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 loco settings where laboratory facilities are often lacking.

Acknowledgements

Funding was partially provided by the Ministry of Health, Myanmar, United Nations Development Program (UNDP) Myanmar, and Saliva Diagnostic Systems, Inc., Vancouver, WA, USA.

Drs Zaw, Frencha and Oo have no financial or other connections with Saliva Diagnostic Systems, Inc. Ms. Ikesh was formerly employed by Saliva Diagnostic Systems, and still owns stock in the company.

References


Masa H, Mibutsu MT, Makura E et al. (1997) Evaluation of rapid on-site clinical HIV test, combined with counselling. AIDS 11, 410-413.


Soto HC, Grenade TC, Nater CA et al. (1997) Field evaluation of rapid HIV serologic tests for screening and confirming HIV1 infection in Honduras. AIDS 11, 369–375.