Evaluation of rapid HIV testing strategies in under equipped laboratories in the Central African Republic

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Abstract

Voluntary testing is described as being cornerstone to impact the spread of human immunodeficiency virus (HIV) infection if the person who tests positive is counseled. Therefore, simple, accurate and affordable diagnostic tests are required. The immunoblot test used in developed countries is too expensive for large-scale use in developing countries. Therefore, alternative strategies must be developed.

A strategy based on two consecutive rapid tests was tested. This strategy used the Determine HIV-1/2® (Abbott Laboratories®, Tokyo, Japan) rapid immunochromatographic test as a screening test and the Uni-Gold HIV test® (Trinity Biotech®, Dublin, Ireland), SDHO HIV 1/2 test® (SDHO laboratories®, Saint-Sauveur des Monts, Canada), HIV 1/2 Quick test® (Cypress Diagnostics®, Langdorp, Belgium) or Retrocheck HIV® test (Qualpro Diagnostics®, Goa, India) as a confirmatory test. Reference serum samples (HIV-positive and HIV-negative) were first used to evaluate the four confirmatory tests. Secondly, 159 serum samples were used to compare the “consecutive” testing strategy used in our laboratory with the two-test strategy. Thirdly, we tested the feasibility of using this two-test strategy in an under equipped laboratory.

The sensitivity and negative predictive value of both test strategies were 100%. The specificity and positive predictive value of the four confirmatory tests were similar (>98%). The strategy used in our laboratory and the two-test strategy always gave identical results, regardless of where this strategy was performed (Institut Pasteur de Bangui or M’baïki hospital).

This new strategy appears to be reliable, simple, feasible and rapid in under equipped laboratories. It allows counseling and results to be given on the same day, which should improve post-test counseling.

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1. Introduction

Voluntary testing is described as being cornerstone to impact the spread of human immunodeficiency virus (HIV) infection if the person who tests positive is counseled. However, in Bangui, the capital of the Central African Republic (CAR), where 16.9% of individuals aged between 25 and 29 years are estimated to be HIV seropositive (Matsika-Claquin et al., 2004), only 15,000 HIV tests are performed annually for a population of approximately 700,000 inhabitants (2%). This is partly because HIV diagnosis is too expensive for most of the population.

It is thus of utmost importance to develop simple, accurate and affordable assays for the detection of HIV antibodies in individuals suspected of being HIV seropositive so that they can be informed and receive counseling.

Advantages of consecutive testing based on WHO strategy II (WHO, 1992 and 1997) using the mixed automatic EIA test Vidas HIV DUO® (BioMérieux Laboratories®),
Marcy l’Étoile, France) as a screening test and the Determine HIV-1/2® (Abbott Laboratories®, Tokyo, Japan) rapid immunochromatographic test for confirmation have been previously described (Ménard et al., 2003). This strategy gave excellent results and is now routinely used at the Institut Pasteur de Bangui. However, the Vidas HIV DUO® test cannot be performed in all laboratories, especially in under equipped laboratories as it requires a Mini Vidas® or Vidas® analyzer.

Alternative strategies that use a combination of simple and rapid enzyme-based assays for screening and confirmation have been shown to be as sensitive and specific as the initial screening assay followed by western blotting analysis (Spielberg et al., 1990; Fonseca and Anand, 1991; Mitchell et al., 1991; van der Groen et al., 1991; Béchets et al., 1992; Nkengasong et al., 1992; Mortimer, 1992; Urassa et al., 1992; WHO, 1992; Nunn et al., 1993; Urassa et al., 1994; Thorstensson et al., 1995; Carvalho et al., 1996; Itriravong et al., 1996).

Eight centers for HIV counseling and testing will be constructed in the CAR at the end of 2004 with the aid of the Global Fund; only a strategy based on two rapid tests can be used in these centers.

Therefore, the Ministry of Health of the CAR has decided to adopt a strategy using two consecutive rapid tests that can be performed in under equipped laboratories.

The aim of this study was to evaluate the accuracy of a consecutive testing strategy based on strategy II of WHO, using two rapid tests. We chose Determine HIV-1/2® test (Abbott Laboratories®, Tokyo, Japan) as a screening test. This test had a sensitivity and a negative predictive value of 100% in a previous study conducted on sera from Centrafrican patients (Ménard et al., 2003). We chose four different confirmatory tests: Uni-Gold HIV test® (Trinity Biotech®, Dublin, Ireland), SDHO HIV 1/2 test® (SDHO laboratories®, Saint-Sauveur des Monts, Canada), HIV 1/2 Quick test® (Cypress Diagnostics®, Langdorf, Belgium) and Retrocheck HIV® (Qualpro Diagnostics®, Goa, India). These tests are rapid immunochromatographic tests (antigen source: recombinant peptides, storage temperature: 2–30 °C). The Uni-Gold HIV test® requires two drops of patient serum and two drops of sample running buffer; the SDHO HIV 1/2 test® requires two drops of patient serum and four drops of sample running buffer; the HIV 1/2 Quick test® requires 5 µl of serum and three drops of running buffer and the Retrocheck HIV® test requires one drop of serum and five drops of running buffer. The HIV 1/2 Quick test® takes 10 min and the others take 15 min. As recommended by the manufacturers, samples were considered negative if no red bar appeared in the patient window. When a red bar was visible in the patient window, the sample was considered to be positive.

We compared the results obtained with the consecutive testing strategy used in our laboratory and with the strategy using two rapid tests. Our consecutive testing strategy has been described previously (Ménard et al., 2003). It is based on the use of the Vidas HIV DUO® test (BioMérieux laboratories®, Marcy l’Étoile, France) as a screening test and the Determine HIV-1/2® test (Abbott Laboratories®, Tokyo, Japan) as a confirmatory test. We tested 159 serum samples taken from patients above two years old, who attended the Institut Pasteur de Bangui for an HIV test with both strategies. Serum samples that were non reactive with the screening test were confirmed using all confirmatory tests. Those reactive with both methods were considered to be HIV-positive. Those that were reactive any red color was visible in the patient window, the sample was considered to be positive.

The following tests were used to confirm positive results obtained with the first test: Uni-Gold HIV test® (Trinity Biotech®, Dublin, Ireland), SDHO HIV 1/2 test® (SDHO laboratories®, Saint-Sauveur des Monts, Canada), HIV 1/2 Quick test® (Cypress Diagnostics®, Langdorf, Belgium) and Retrocheck HIV® (Qualpro Diagnostics®, Goa, India). These tests are rapid immunochromatographic tests (antigen source: recombinant peptides, storage temperature: 2–30 °C). The Uni-Gold HIV test® requires two drops of patient serum and two drops of sample running buffer; the SDHO HIV 1/2 test® requires two drops of patient serum and four drops of sample running buffer; the HIV 1/2 Quick test® requires 5 µl of serum and three drops of running buffer and the Retrocheck HIV® test requires one drop of serum and five drops of running buffer. The HIV 1/2 Quick test® takes 10 min and the others take 15 min. As recommended by the manufacturers, samples were considered negative if no red bar appeared in the patient window. When a red bar was visible in the patient window, the sample was considered to be positive.

2.2. Evaluation of HIV diagnostic tests

We used a collection of 286 reference serum samples that had been stored at −80 °C to evaluate the sensitivity, specificity, negative predictive value and positive predictive value of the four confirmatory tests (173 negative serum samples and 113 positive serum samples, 56 from AIDS patients with low CD4 counts and 57 from HIV positive serology patients with high CD4 counts). The HIV status of reference serum samples had previously been determined using two EIA tests: GenelaviX Mix® (Sanofi Diagnostic Pasteur®, Marnes la Coquette, France), and Vironostika HIV Uni-Form plus O® (Organon technik®, Boxtel, the Netherlands). Positive samples were confirmed by western blotting (New Lav Blot®19, BioRad®, Marnes la Coquette, France).

2.3. Evaluation of the strategy using two consecutive rapid tests at Institut Pasteur de Bangui

2.3.1. Description of the HIV diagnostic tests

The first test was the Determine HIV-1/2® test (Abbott Laboratories®, Tokyo, Japan), a rapid immunochromatographic test (antigen source: combined recombinant and synthetic peptides, storage temperature: 2–30 °C). This test requires 50 µl of serum and takes 15 min. As recommended by the manufacturer, the patient was considered to be HIV-negative if there was no red bar in the patient window. When any red color was visible in the patient window, the sample was considered to be positive.
Fig. 1. Consecutive testing strategy based on WHO strategy II using two rapid tests.

with the screening test and negative with the confirmatory test were retested by both methods. If repeatedly discrepant results were obtained, the patient was asked to give a second sample 2–4 weeks later. When the serum sample became negative with the screening test or the tests gave the same results as previously, the patient was considered HIV-negative. If the confirmatory test was positive, the patient was considered HIV-positive. If the confirmatory test was negative, the patient was asked to give another sample 2–4 weeks later (Fig. 1).

2.4. Evaluation of the strategy using two consecutive rapid tests in under equipped laboratory

To evaluate the feasibility of using the strategy based on two rapid test in under equipped laboratories, this strategy was used to test 194 serum samples taken from patients who attended the M’baïki hospital, a small town located 100 km from Bangui. This hospital has electricity only for surgical interventions. Therefore, the tests are stored in laboratory at room temperature, which is often around 30°C. All serum samples were stored at +4°C in a refrigerator usually used for vaccines and working with petrol, and were sent weekly to the Institut Pasteur de Bangui at +4°C in ice box where they were tested using the Vidas HIV DUO® and the Determine HIV-1/2® test strategy.

2.5. Ethical approval

As there is no national ethical committee in the CAR, this study was approved by the expert committee from the Ministry of Health in CAR. Written informed consent was obtained from all patients.

3. Results

3.1. Evaluation of HIV diagnostic tests

We used the 286 reference serum samples (173 negative serum samples and 113 positive serum samples, 56 from AIDS patients with low CD4 counts and 57 from HIV positive serology patients with high CD4 counts) to evaluate the SDHO HIV 1/2 test® test, only 248 available reference serum samples (145 negative serum samples and 103 positive serum samples, 48 from AIDS patients with low CD4 counts and 55 from HIV positive serology patients with high CD4 counts) to evaluate the Retrocheck HIV® test, 190 available reference serum samples (110 negative serum samples and 80 positive serum samples, 38 from AIDS patients with low CD4 counts and 42 from HIV positive serology patients with high CD4 counts) to evaluate the HIV 1/2 Quick test® test and 119 available reference serum samples (68 negative serum samples and 51 positive serum samples, 23 from AIDS patients with low CD4 counts and 28 from HIV positive serology patients with high CD4 counts) to evaluate the Uni-Gold HIV test® test. The results obtained with the tests are summarized in Table 1.

3.2. Evaluation of the strategy using two consecutive rapid tests at Institut Pasteur de Bangui

The results obtained using the consecutive two test strategy with 159 serum samples from patients taking HIV tests are presented in Table 2. The concordance between the strategy used at the Institut Pasteur de Bangui and that using two rapid tests was 100%. However, five patients were considered as indeterminate with the first serum sample (discordant results: positive with Vidas HIV DUO® and negative with
Table 1
Results of the Uni-Gold HIV®, SDHO HIV 1/2®, HIV 1/2 Quick® and Retrocheck HIV® tests with reference serum samples.

<table>
<thead>
<tr>
<th>Assays</th>
<th>No. of samples with the following results</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% Positive predictive value (95% CI)</th>
<th>% Negative predictive value (95% CI)</th>
<th>% Accuracy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total True positive</td>
<td>119</td>
<td>68 (94.2–100)</td>
<td>98.5 (93.1–99.9)</td>
<td>98.0 (90.7–99.9)</td>
<td>100 (95.7–100)</td>
<td>99.2 (95.9–99.9)</td>
</tr>
<tr>
<td>False positive</td>
<td>50</td>
<td>1 (97.3–100)</td>
<td>99.3 (97.7–99.9)</td>
<td>100 (98.4–100)</td>
<td>99.3 (97.7–99.9)</td>
<td>98.8 (96.7–99.7)</td>
</tr>
<tr>
<td>True negative</td>
<td>68</td>
<td>173 (98.9–100)</td>
<td>100 (97.3–100)</td>
<td>100 (96.3–100)</td>
<td>100 (98.3–100)</td>
<td>100 (98.0–100)</td>
</tr>
<tr>
<td>False negative</td>
<td>0</td>
<td>0 (99.9–100)</td>
<td>100 (99.9–100)</td>
<td>100 (99.9–100)</td>
<td>100 (99.9–100)</td>
<td>100 (99.9–100)</td>
</tr>
</tbody>
</table>

Table 2
Comparison of results obtained with the Vidas HIV DUO® and Determine HIV-1/2® strategy and those obtained with Determine HIV-1/2® or Uni-Gold HIV® test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® or Retrocheck HIV® strategy with serum samples from patients undergoing an HIV test at the Pasteur Institute of Bangui.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Vidas HIV DUO® + Determine HIV-1/2® or HIV-1/2® Quick® or Retrocheck HIV® tests</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>38</td>
<td>0</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>121</td>
<td>121</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>0</td>
<td>159</td>
<td></td>
</tr>
</tbody>
</table>

Note: Five patients were considered as indeterminate initially (Vidas HIV DUO® positive and Determine HIV-1/2® negative) and considered as negative after retesting 3 weeks later.

3.3. Evaluation of the strategy using two consecutive rapid tests in under equipped laboratory

We next evaluated the feasibility of using the rapid testing strategy in under equipped laboratories (Table 3). The concordance between the strategy performed at the Institut Pasteur de Bangui and that using two rapid tests performed at the M’baiki hospital on 194 sera was 100%. However, six patients were considered as indeterminate with the strategy performed at the Institut Pasteur de Bangui with the first serum sample (discordant results: positive with Vidas HIV DUO® and negative with Determine HIV-1/2®) and considered as negative with the second serum sample collected 3 weeks later (negative with Vidas HIV DUO®) and two patients were considered as indeterminate with the strategy performed at the M’baiki hospital with the second serum sample collected 3 weeks later (positive with Vidas HIV DUO®) and two patients were considered as indeterminate with the strategy performed at the M’baiki hospital.

Table 3
Comparison of results obtained with the Determine HIV-1/2® or Uni-Gold HIV® test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® or Retrocheck HIV® strategy performed at the Pasteur Institute of Bangui with serum samples from patients undergoing HIV tests at M’baiki hospital.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Determine HIV-1/2® or Uni-Gold HIV® test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® or Retrocheck HIV® tests</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>35</td>
<td>0</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>169</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>169</td>
<td>194</td>
<td></td>
</tr>
</tbody>
</table>

Note: Six patients were considered as indeterminate initially (Determine HIV-1/2® positive and Uni-Gold HIV® test® or SDHO HIV 1/2 test®, HIV 1/2 Quick test® or Retrocheck HIV® negative) and considered as negative after retesting 3 weeks later.
M’baiki hospital with the first serum sample (discordant results: positive with Determine HIV-1/2® and negative with Uni-Gold HIV test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® and Retrocheck HIV®) and considered as negative with the second serum sample collected 3 weeks later (negative with Determine HIV-1/2®).

4. Discussion

The Ministry of Health of the CAR has decided to adopt the WHO strategy II for HIV diagnosis in 2003. In the small number of reference laboratories in Bangui that are equipped with material and electric facilities, screening is based on ELISA and confirmation is based on either ELISA or rapid tests. In other areas of the country where electric facilities are rarely available and in under equipped laboratories in Bangui, the same consecutive testing strategy using two rapid tests has been adopted for practical and economic reasons; this is particularly true in HIV testing and counseling centers. The Determine HIV-1/2® test was chosen as a screening test because of its proven performance in many developing countries: Honduras and the Dominican Republic (Palmer et al., 1999), Thailand (Arai et al., 1999), Vietnam (Lien et al., 2000), Tanzania (Urassa et al., 1994) and the CAR (Ménard et al., 2003). All of these studies reported that the Determine HIV-1/2® test is 100% sensitive and two reported 100% specificity (Palmer et al., 1999; Arai et al., 1999).

We evaluated four different potential confirmatory tests: Uni-Gold HIV test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® and Retrocheck HIV® tests. We tested the Uni-Gold HIV test® on a smaller number of sera because it has previously been evaluated in other countries and only needed to be confirmed in the CAR (Constantine NT, 2003; Giles RE, 1999). In contrast, we found no references to the SDHO HIV 1/2 test®, HIV 1/2 Quick® and Retrocheck HIV® tests and therefore we assessed their performances on a larger number of available reference serum. All four tests gave satisfactory results with 100% sensitivity and more than 98% specificity.

The concordance between the previously described strategy used at the Institut Pasteur de Bangui, and that using two rapid tests was 100%, whereas the rapid test strategy was performed (Institut Pasteur de Bangui or the M’baiki hospital). Therefore, this two-test strategy appears to be reliable, simple and feasible in under equipped laboratories. It can be used for one sample at a time, avoiding the need to wait for a large number of samples as with classic EIA tests on microplates. This prevents errors due to the inversion of samples or well-to-well contamination. The results can be given to the patient on the same day. This is very important because recent studies performed in Africa indicated that volunteers for HIV testing preferred receiving counseling and results on the same day. This same-day testing format improves post-test counseling rates (McKenna et al., 1997; Bakari et al., 2000; Keenan and Keenan, 2001). The only problem with the alternative strategies is that there is still a possibility that a sample found to be positive by two tests might actually be negative. This risk was estimated to be 1.5–2 per 10,000 in our algorithm, considering the specificity of each test. The only means of avoiding this is to perform western blotting on each positive sample, which is impossible in developing countries especially when the HIV seroprevalence is so high. However, recent studies have shown that samples that are repeatedly reactive in sequential antibody screening assays but which are western blotting negative should be interpreted with caution because some HIV-1 antibody assays are reactive earlier in the infection process than western blotting (Zaaijer et al., 1992; Tamashiro et al., 1993). The best option would be to repeat the tests on another sample taken 2–4 weeks later, as in our algorithm.

The strategy based on Determine HIV-1/2® plus Uni-Gold HIV test®, SDHO HIV 1/2 test®, HIV 1/2 Quick test® or Retrocheck HIV® all have quite similar performances. All tests are easy to perform and can be stored at between 4 and 30 °C. Therefore, the main factors that need to be taken into account by the Ministry of Health of the CAR when choosing tests are the price and the easy delivery.

In conclusion, the new algorithm based on two rapid tests (Fig. 1) is very efficient for the diagnosis of HIV infection in developing countries; it is both reliable and low cost.

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References


