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Advantages of an alternative strategy based on consecutive HIV serological tests for detection of HIV antibodies in Central African Republic

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Abstract

Voluntary testing and counselling are accepted widely for the prevention of human immunodeficiency virus (HIV) infection. Therefore, simple, accurate and affordable tests are required. The diagnosis strategy used in developed countries, based on an immunoblot confirmatory test, cannot be used on a large scale in developing countries because of its cost. Therefore, alternative strategies must be developed. In this study, we tested according UNAIDS and World Health Organisation recommendations for HIV testing strategies, a strategy based on two consecutive tests, using the mixed automatic enzyme immunoassays test Vidas HIV DUO© as a screening test and Determine Abbott© rapid immunochromatographic test as a confirmatory test. In first step, reference serum samples (113 HIV-positive and 167 HIV-negative) were used to evaluate the performance of both tests. In a second step, 876 serum samples from patients were used to compare the 'simultaneous' testing strategy currently used in Central African Republic (CAR) to the 'consecutive' testing strategy. The sensitivity and negative predictive value of both tests were 100%. The specificity and positive predictive value of Determine Abbott© ($>99\%$) were higher than those of Vidas HIV DUO© (90.4 and 87.6%, respectively). In all cases in which the two tests gave discrepant results, the patient was considered HIV-negative after a second test carried out 2–4 weeks later since the optical density value of the Vidas HIV DUO© of the second sample was not higher than that of the first sample. This new consecutive testing strategy appears to be reliable, simple and rapid, allowing counselling and results to be given on the same day, which we believe is important for improving post-test counselling. Furthermore, the consecutive testing strategy reduces the cost of testing, which is very important in developing countries.

Keywords: HIV-1; HIV-2; Algorithm; HIV antibodies testing; Consecutive testing strategy; Serological test; Africa; Central African Republic

1. Introduction

Voluntary testing and counselling are described as being cornerstones of human immunodeficiency virus (HIV) prevention strategies and of strategies for caring for HIV-infected individuals. However, in Bangui, the capital of the Central African Republic (CAR), where the HIV seroprevalence is estimated to be about 14% in individuals aged between 15 and 49 years (Pison, 2000), only 15 000 HIV testings are performed annually for a population of approximately 700 000 inhabitants (2%). This is partly because the cost of HIV diagnosis is too high for most of the population.

The importance of 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 counselling cannot be stressed enough. The use of Western blot (WB) assays has been limited by the high costs, the need for well trained manpower, the lack of a consensus concerning interpretation criteria and the presence of indeterminate WB

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results, especially in the case of African serum samples (Tamashiro et al., 1993). Alternative strategies that use a combination of a simple and rapid enzyme immunoassays (EIA) and agglutination tests have been evaluated in various settings and have been shown to be as sensitive and specific as the initial screening assay followed by WB analysis (Spielberg et al., 1990; Fonseca and Anand, 1991; Mitchell et al., 1991; Van der Groen et al., 1991; Behets 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; Ittiravivongs et al., 1996). The strategy used for laboratory diagnosis of HIV infection in CAR is a compromise between the conventional strategies used in developed countries and the alternative strategies recommended by the World Health Organisation (WHO) (WHO, 1992, 1997). This strategy includes screening for virus-specific antibodies by simultaneously using two EIA tests. HIV antibody-positive samples are usually retested using a WB or a third EIA test. The WHO has recommended a number of strategies for HIV testing, both for diagnosis purposes and for estimating the seroprevalence (WHO, 1997). The WHO strategy II is based on two consecutive tests, for the diagnosis of HIV infection. The first test must be very sensitive so that a non-reactive sample can be considered to be HIV-negative. A reactive sample must then be tested with a second more specific test. A sample found to be positive by both tests is considered to be HIV-positive. A patient found to be positive in the first test and negative with the second test is indeterminate and the same sample must be retested by both methods. If the same result is found, the patient must give a second test sample 2–4 weeks later. This strategy is recommended for the diagnosis of asymptomatic individuals in populations in which the HIV seroprevalence is above 10%.

The aim of this study was to evaluate the accuracy and the advantages of the consecutive testings strategy according UNAIDS and WHO recommendations for the diagnosis of HIV infection in CAR.

2. Materials and methods

This study was performed at the Pasteur Institute of Bangui between October 2001 and September 2002. The algorithm evaluated in this study, is derived from strategy II of the WHO (WHO, 1997).

2.1. Description of the HIV diagnosis tests

The first test consisted of the mixed automatic EIA test Vidas HIV DUO© (antigen source: three synthetic peptides and three monoclonal anti-p24 antibodies, storage temperature: 2–8 °C) (BioMérieux laboratories, Marcy l'Etoile, France). This test requires a Mini Vidas© or Vidas© analyser, 200-μl serum and lasts for 100 min. The principle of the test combines 2 EIA reactions with a final fluorescent detection (ELFA). The solid phase receptacle serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and are pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. Results of the optical density (OD) were interpreted as recommended by the manufacturer: $OD < 0.25$: samples are considered to be negative, $0.25 \leq OD < 0.35$: samples are considered to be borderline positive, $OD \geq 0.35$, samples are considered to be positive.

The second test used to confirm positive results obtained with the first test was the Determine Abbott© rapid immunochromatographic test (antigen source: combined recombinant and synthetic peptides, storage temperature: 2–30 °C) (Abbott Laboratories, Tokyo, Japan). This test requires 50 μl serum and lasts for 15 min. As recommended by the manufacturer, in the absence of a red bar in the patient window, sample was considered to be negative. When any red color was visible in the patient window, the sample was considered to be positive.

2.2. Evaluation of HIV diagnosis tests

To evaluate the sensitivity, specificity, negative predictive value and positive predictive value of the Vidas HIV DUO© and Determine Abbott© tests, a collection of reference serum samples that had been stored at −80 °C was used. The HIV status had been determined previously for these serum samples using two EIA tests: Génélavia Mixt© (Sanofi Diagnostic Pasteur, Marne la Coquette, France), and Vironostika HIV Uni-Form plus O© (Organon technika, Boxtel, the Netherlands). Positive samples were confirmed using a WB test (New Lav Blot I©, BioRad, Marne la Coquette, France).

2.3. Evaluation of the consecutive testing strategy

In a second step, we compared the results obtained with the simultaneous testing strategy currently used in CAR to our consecutive testing strategy. For this study, 876 serum samples taken from patients who attended the Pasteur Institute of Bangui for an HIV test were systematically tested using both tests (Vidas HIV DUO© and Determine Abbott©). Serum samples that were reactive with both methods were considered to be HIV-positive. Those that were non-reactive with both methods were considered to be negative. Those that were reactive with one test and negative with the other test were retested by both methods. If repeatedly discrepant results were obtained, the patient was asked to take second sample 2–4 weeks later. When the OD of

the Vidas HIV DUO© test did not increase in the second sample the patient was considered as HIV-negative. When the OD increased, the second sample was also tested with the Determine test. If the Determine test was positive, the patient was considered as HIV-positive otherwise asked to take another sample 2–4 weeks later (Fig. 1).

3. Results

We evaluated the Vidas HIV DUO© and Determine Abbott© tests using 113 positive serum samples (46 symptomatic patients) and 167 negative serum samples (Table 1). The Vidas HIV DUO© showed a sensitivity and a negative predictive value of 100%; however, 16 false positives were found, thus the specificity and the predictive positive value were only 90.4 and 87.6%, respectively. The sensitivity and the negative predictive value of the Determine Abbott© test were also 100%, but its specificity and positive predictive value were much higher (> 99%).

Results of the evaluation of the consecutive testing strategy with 876 serum samples from patients taking HIV tests is presented in the Table 2. A total of 830 (94.7%) gave similar results with both tests and 46 (5.3%) gave discrepant results:

- of the 537 samples found to be HIV-negative by the Vidas HIV DUO© test, 532 were negative and 5 were positive according to the Determine Abbott© test,
- of the 326 samples found to be positive with the Vidas HIV DUO© test, 298 were positive and 28 were negative according to Determine Abbott©,
- of the 13 samples that gave borderline positive results with Vidas HIV DUO©, 11 were negative and 2 were positive according to the Determine Abbott© test.

Of the 46 samples that gave discrepant results, the results of the second tests performed on the same day were identical in 45 cases (97.8%). Only one sample that was borderline positive with Vidas HIV DUO© became negative. All patients with serum samples presenting persistent discrepancies between the two tests, second serum samples were retested between 2 and 4 weeks later. For all of them (45/45), the OD of the Vidas HIV DUO© test did not increase in the second sample, therefore all were considered HIV-negative when our algorithm was used.

4. Discussion

We wanted to evaluate a new strategy and new tests in our laboratory since our experience of the past years with microplates ELISA tests was not totally satisfactory. Many reasons lead us to change. First, microplates ELISA tests are time consuming; second, sera were only tested twice a week for economical and practical reasons and patients had to wait at least 3 days to get their result; third, many discrepant results were observed with the two tests used (Genelavia Mixt© and Vironostika

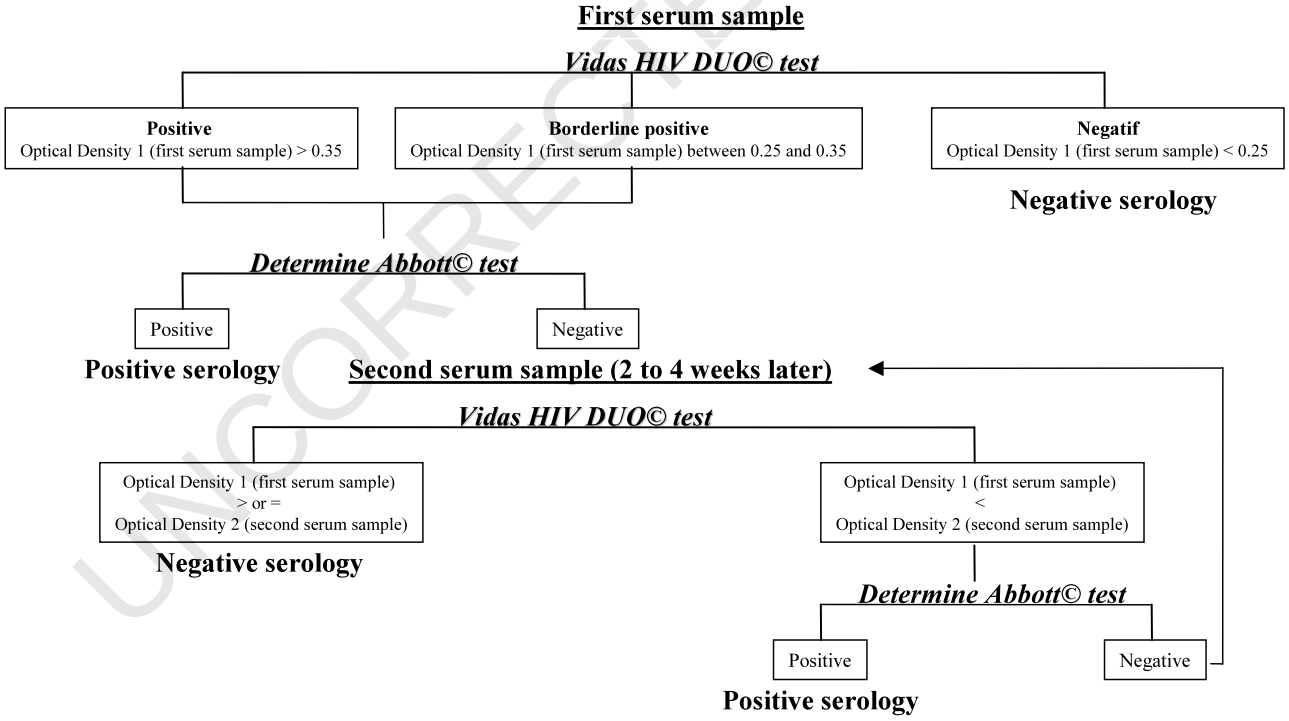

Fig. 1. Consecutive testing strategy used at the Pasteur Institute of Bangui based to the strategy II of the WHO.

Table 1
Results of the evaluation of Vidas HIV DUO© and Determine Abbott© with 280 reference serum samples for HIV infection

Assays	No. of samples with the following results					% Sensitivity (95% CI)	% Specificity (95% CI)	% Positive predictive value (95% CI)	% Negative predictive value (95% CI)	% Accuracy (95% CI)
	Total	True positive	False positive	True negative	False negative					
Vidas HIV DUO©	280	113	16	151	0	100	90.4	87.6	100	94.3
						(95.9–100)	(87.4–94.2)	(80.4–92.5)	(96.9–100)	(90.7–96.6)
Determine Abbott©	280	113	1	166	0	100	99.4	99.1	100	99.6
						(95.9–100)	(96.2–100)	(94.5–100)	(97.2–100)	(97.7–100)

Table 2
Results of the evaluation of the algorithm with 876 serum samples from patients presenting for the diagnosis of HIV infection

Assays	Vidas HIV DUO©			Total
	Positive	Borderline positive	Negative	
Determine Abbott©				
Positive	298	2	5	305
Negative	28	11	532	571
Total	326	13	537	876

HIV Uni-Form plus O©). Furthermore, two sera presented false negative results with the Vironostika test, and we did not want to use this test anymore. The Genelavia Mixt© has been replaced with the Genscreen test (Biorad, Marne la Coquette) since the year 2001 and this new test gave a lot of false positive results in our laboratory. Its specificity has been evaluated at 93% in a recent study conducted in our laboratory on more than 6000 sera.

The Vidas HIV DUO©, despite its cost (3.60 US $), which is higher than most other similar tests (2–3 US $), and the fact that it needs Mini Vidas© or Vidas© analysers presented some advantages as an alternative to microplates ELISA tests. As a Mixt EIA test, it allows the early diagnosis of HIV infection by simultaneously testing for the p24 antigen and antibodies to HIV-1 and 2. The simplicity and the reliability of the automated equipment, which needs little maintenance, make it particularly suitable for the conditions found in Bangui. It can be used for one sample at a time. Therefore, there is no need to wait for a large number of samples as with classic EIA tests on microplates, which prevents errors due to the inversion of samples or well to well contaminations; and the results can be given to the patient on the same day. This test can be used in any laboratory with a correct electrical installation which is the case of our laboratory. However, this test appeared to present some disadvantages that were not expected considering the performance declared by the manufacturers, a sensitivity of 100% and a specificity of more than 99% (Laperche et al., 2001). The low specificity of this test when used on sera from CAR (90.4% in the evaluation of the tests and 93.9% in the evaluation of our algorithm) is clearly a major handicap for its recommendation as a first step test in Africa. However this bad performance is not very different from that observed with microplate ELISA tests evaluated in our laboratory on similar sera, and it is counterbalanced by some advantages: (i) with a negative predictive value of 100%, all negative results can be considered as being real negatives which is essential for a first step test; (ii) the Vidas HIV DUO test gives quantitative results, on the contrary of rapid tests, and most false positive results had very low OD values. Had the bordeline positive

results been considered as negative, the specificity would have been of 94.6% with reference samples and 95.1% with the 876 samples used in the evaluation of the strategy and, had all sera with an OD value of less 3 considered as negative, the specificity would have reached 96.4% with reference samples and 98.6% with the 876 samples of the evaluation of the strategy; (iii) recently in our laboratory this test detected a recent seroconversion that was not detected with the Determine Abbott© test. We consider that for a reference laboratory such as the Pasteur Institute of Bangui for CAR, it is important to use a quantitative test and not only rapid qualitative tests. The interpretation of the OD value and the determination of the cut-off value of this test will probably need to be adapted to the condition of CAR from our experience with this test in some months.

Therefore, despite its low specificity we chose to keep the Vidas HIV DUO as a first step test of the consecutive testing strategy. Of course, because of the high number of false positive results, all positive results have to be confirmed by a second test with different characteristics. The Determine Abbott© test was chosen because of its sensitivity and specificity. The Determine assay has been assessed in four developing countries: Honduras and the Dominican Republic (Palmer et al., 1999), Thailand (Arai et al., 1999), Vietnam (Lien et al., 2000) and Tanzania (Urassa et al., 1994). Two of these studies reported a 100% specificity for the Determine assay (Palmer et al., 1999; Arai et al., 1999). Its advantages are its reliability, low cost (2.8 US $), easy storage (2–25 °C) and simplicity. We wished to use a rapid test so that the results can be given to the patient within a few hours. This is very important because recent studies performed in Africa indicated that volunteers for HIV testing preferred receiving counselling and the results of the HIV tests on the same day. This same day testing format improves post-test counselling rates (Mc Kenna et al., 1997; Bakari et al., 2000; Keenan and Keenan, 2001).

In our study, there was no difference between our consecutive testing strategy and simultaneous testing with both tests currently used in CAR. 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 HIV-negative. This risk was evaluated at 8 per 10 000 in our algorithm considering the specificity of each test. However, this risk was identical with simultaneous testing. The only means of avoiding this is to perform a WB test 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 WB negative should be interpreted with caution because some HIV-1 antibody assays are reactive earlier in the infection process than WB (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 main advantage of such strategies is that they reduce the cost of the HIV diagnosis, which is essential in developing countries. We estimated the cost of different strategies using Vidas HIV DUO© and Determine Abbott©. The evaluation has been made for 100 patients with an HIV estimated prevalence rate of 15 and 5.3% discordant sera. The cost for the strategy used in developed countries (two simultaneous EIA tests and a confirmatory WB) is 13 US $ per patient; the cost for the strategy using two simultaneous EIA tests and a retesting on a second sample is 6.80 US $ (−47%); the cost of the strategy II of the WHO is 4.80 US $. As the reproducibility of both tests was excellent, the advantage of retesting discrepant serum samples on the same day is very small. To reduce the cost of the test, we propose that patients presenting discrepant results are retested 2–4 weeks later (Fig. 1); with our algorithm, the cost drops to 4.50 US $ (−65%).

In conclusion, our new algorithm, proposed in Fig. 1, is very efficient for the diagnosis of HIV infection; it provides a reliable, low cost test. However, the specificity of the first step test should be better and it would be important that before being commercialised in Africa all tests would be evaluated with African sera to avoid this type of disagreement. Using a first step test of 99% the cost of our algorithm would drop to 4.11 US $.

Due to the cost of the analyser and to the fact that electrical facilities do not exist in most parts of the country, another algorithm based on two rapid tests will be proposed in the near future for the other parts of the country with reduced technical means.

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