PD-AAE-114

Field Evaluation of Rapid Assays For HIV in Zaire

Project Manager: Dr. Duale Sambe

Principal Investigator: Dr. K. Milenge

Laboratory Consultant: Claire K. Mulanga

This project was funded by the U.S. Agency for International Development (USAID) and technical assistance was provided by AIDSTECH, a Division of Family Health International (FHI).

The following organizations and agencies were instrumental in assuring the success of this study: Projet Soins De Sante Primaire En Milieu Rural (SANRU), Bureau Central De Coordination Du Programme De Lutte Contre Le SIDA (BCC), Ministry of Health, Projet SIDA and the following hospitals: Kimpese, Kimbanseke, Karawa, Tshikaji, Vanga.

TABLE OF CONTENTS

I. **Introduction** 1

Rationale 1

Literature Review 1

II. **Objectives** 1

III. **Materials and Methods** 2

Tests Evaluated 2

Field Sites 2

Training 3

Study Design 3

Statistical Comparisons 5

IV. **Results** 7

Screening Tests 7

Supplemental Testing 8

Seroprevalence 9

Proposed HIV Testing Strategy 10

V. **Discussion** 11

Rapid Tests 11

Testing Strategy 12

HIV Counseling 13

VI. **Recommendations** 14

VII. **References** 14

TABLE OF CONTENTS (CON'T)

TABLES

Table 1 4

Table 2 6

Table 3 7

Table 4 7

Table 5 8

Table 6 8

Table 7 9

Table 8 9

Table 9 10

Table 10 11

ZAIRE COUNTRY REPORT

I. Introduction

Rationale

Transfusion of unscreened blood continues to be a major health problem in developing countries. In many of the laboratories in Africa, there are not the technical and/or financial resources available to perform the standard ELISA and Western Blot tests for HIV antibody. Yet the demand for a safe and adequate blood supply remains high.

Even if these tests were obtained, sites where blood is transfused often are not able to support sophisticated systems for large scale testing. Assays are needed which are simple, inexpensive, and less time-consuming than the conventional ELISA and Western Blot.

Literature Review

A number of rapid tests for antibody to HIV have recently become commercially available which meet one or more of the above criteria (1,2,3,4). While a variety of these assays have been evaluated with African sera (5,6,7), the tests were often performed in those labs which were above the country average in amount of necessary equipment, experience in HIV testing, and financial support.

Taking into consideration the advantages of these rapid tests, however, it is essential that they are evaluated within the real-life situations and environments in which they would be the most useful. In the present study, the assays were performed in urban and rural hospitals throughout Zaire.

II. Objectives

1. To evaluate the HIVCHEK(Dupont) as a screening test for detecting antibody to HIV-1 in the field laboratories of Zaire as compared to the ELISA test (Genetic Systems).

2. To evaluate the SERODIA-HIV(Fujirebio) and RETROCELL(Abbott) as supplementary tests for detecting antibody to HIV-1 in the field laboratories of Zaire as compared with the Western Blot.

3. To evaluate a revised HIV testing strategy with HIVCHEK and SERODIA-HIV/RETROCELL as an alternative to the current strategy using ELISA and Western Blot.

III. Materials and Methods

Tests Evaluated

The three tests to be evaluated in this study were chosen on the basis of their running costs, performance in previous studies, commercial availability, and expected usage in Zaire.

The HIVCHEK test is a dot immunobinding assay where the antibody in the test sample is captured by a recombinant protein and absorbed to a nitrocellulose membrane in a test cartridge. Bound antibody is visualized by the addition of a protein A colloidal gold conjugate which gives a reddish color to the membrane when the test is reactive. The test is performed in approximately 5 minutes on fresh serum and can be done in a single sample or small batch format.

The other two tests, SERODIA-HIV and RETROCELL, are agglutination assays. These types of assays are most efficiently used when performed on several specimens in one batch. It is inefficient to run the test procedure for a single specimen. The SERODIA-HIV test is a microtiter agglutination assay in which a diluted serum specimen is mixed with gelatin particles that have been sensitized with partially-purified viral lysate. Particles are agglutinated by the specific antibody to HIV present in the test sample and a settling pattern is observed after a 2 hour incubation period.

The RETROCELL test is a passive hemagglutination assay using the microtiter format. At the time of the study, the test serum was made to react with human red blood cells sensitized with HIV-1 viral lysate and required a 3 hour incubation period. Results are based on visual interpretation of the agglutination pattern.

Field Sites

With the assistance of the National AIDS Committee, 5 sites were chosen to participate in the study. The criteria used for site selection was as follows:

1. Capability to test at least 300 blood samples in 3 months.
2. Capability to test blood donors, persons with symptoms of HIV infection, and individuals practicing high risk behaviors.
3. Having functional facilities for storage of specimens at -20C degrees and at 4C degrees throughout the duration of the study.
4. Accessibility to a reference laboratory capable of performing ELISA and Western Blot tests.

Five laboratories which met the above criteria were selected for participation in this study:

1. Kimbanguiste
2. Kimpese
3. Vanga
4. Tshikaji
5. Karawa

Projet SIDA in Kinshasa served as the reference laboratory for performing ELISA and Western Blot tests for this study.

Training

A standard training module which had been developed at Family Health International for the training of laboratory technicians was used for all field sites. In general, two or three technicians at each site were trained by the principal investigator prior to participation in the study.

Upon completion of training, a panel of 10 sera of known reactivity was tested by every technician on each assay in order to assess their proficiency in performing the test. Panels of serum were also tested once a month to insure technician proficiency and reagent quality.

Of the 15 technicians involved in the study, approximately one-half had received previous training in HIV testing techniques. Average years of laboratory experience for these technicians ranged from 1 to 40 years, with the majority having at least 2-3 years of experience.

Study Design

At the field laboratory, a 10 ml aliquot of blood was received from each person, separated into two vials (Vials "A" and "B"), and labeled with an indentical unlinked specimen code. Vial B was frozen for transport to the reference lab. Vial A was immediately screened by the field lab using the HIVCHEK test, with positive or indeterminate tests being repeated. All positive, indeterminate, and 10% of the negative specimens were then refrigerated and tested in duplicate on SERODIA-HIV and RETROCELL.

At the reference laboratory, Vial B was first screened by ELISA and any positive or indeterminate specimens were tested on Western Blot. All specimens which were positive or indeterminate by HIVCHEK or ELISA and 10% of the HIVCHEK negative specimens were given supplemental testing by Western Blot.

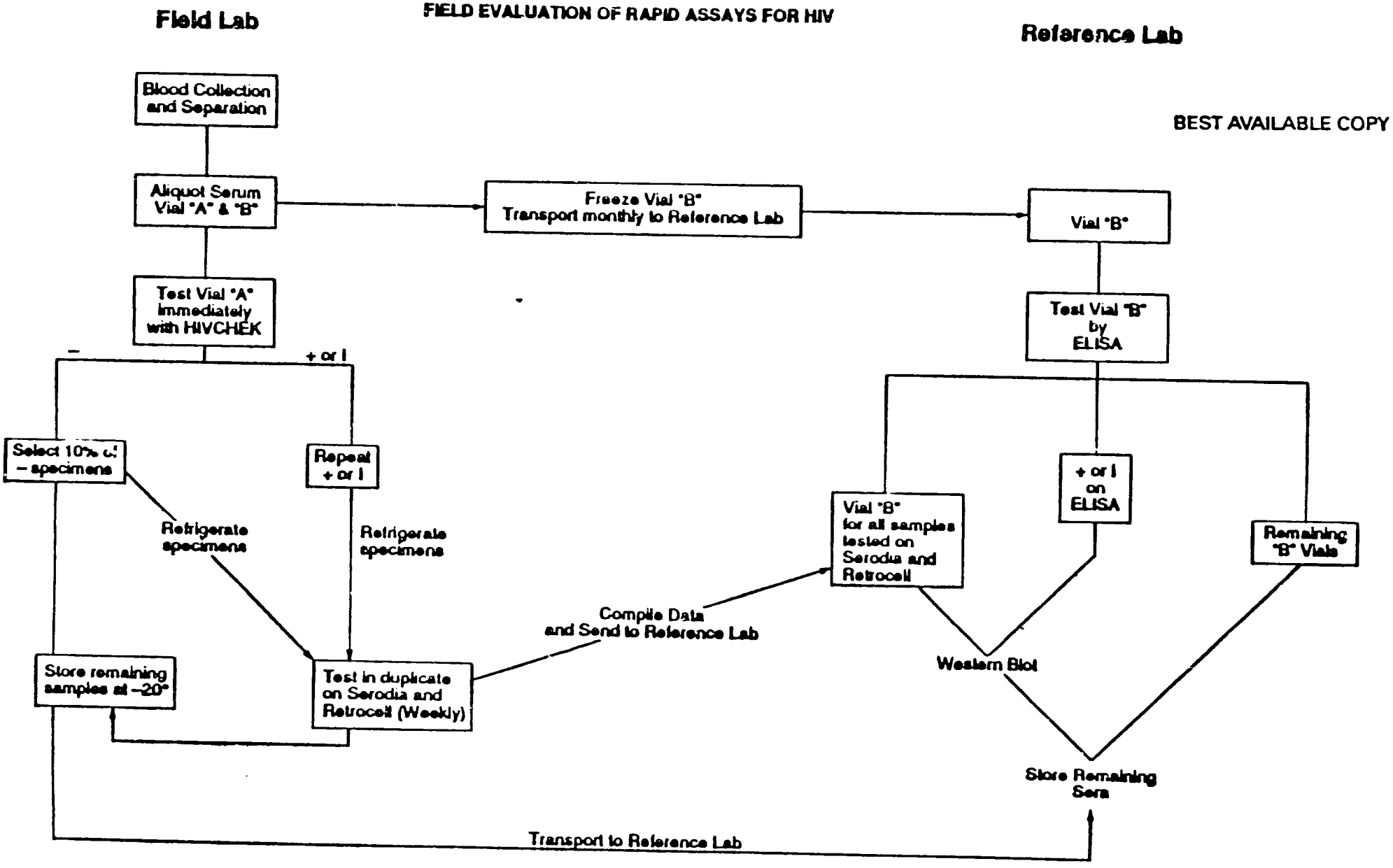
Test results were recorded at each field laboratory on standardized data entry forms and collected at the reference laboratory where they were scanned for accuracy. Copies of all raw data entry forms were then sent to FHI for data analysis.

Table 1

FHI

AIDSTECH

FIELD EVALUATION OF RAPID ASSAYS FOR HIV



BEST AVAILABLE COPY

Specimens were selected from 4 different groups - blood donors, pregnant women, persons at high risk for HIV and persons showing symptoms of AIDS. The purpose for choosing these specimen groups and reporting results by group was to show the effect of differing prevalence rates on the predictive values of the tests. Selection for these groups was not strictly defined and was determined by the director of each study site.

Statistical Comparisons

For the purposes of data analysis, the following definitions were used: A specimen was scored as positive or negative according to the initial results on a given assay. Those with discrepant duplicate results were not scored for that assay. A true positive (TP) specimen was reactive by rapid test (HIVCHEK or SERODIA-HIV or RETROCELL) or ELISA and reactive by Western Blot. A true negative (TN) specimen was nonreactive by any rapid test or ELISA and nonreactive by Western Blot. However, a specimen was also considered as true negative where there was no Western Blot performed, but any of the rapid tests were nonreactive as well as ELISA. A false positive (FP) specimen was reactive by any rapid test or ELISA and nonreactive by Western Blot. A false negative (FN) specimen was nonreative by any rapid assay or ELISA and reactive by Western Blot. Due to the design of the study, specimens which were negative on the HIVCHEK and not part of the 10% negative HIVCHEK tested on the agglutination assays, but were positive by ELISA were excluded from the analysis. In that results were compared to Western Blot as the standard and also that an equal 10% of the ELISA negatives were not blotted, it was decided in council with other investigators that the false negative data for the HIVCHEK would best be presented in this manner.

Table 2 shows the formulas for data analysis based on the comparison to Western Blot for HIV-1. The sensitivity value is the probability that an individual with antibody to HIV will be reactive on a given assay, while the specificity value is the probability that an individual who does not have antibody to HIV will be nonreactive on a given assay. The negative predictive value is the probability that antibody to HIV is not present when the test is negative and the positive predictive value is the probability that a positive test result is indicative of HIV infection.

Table (2): Formulas Used For Data Analysis Based on Comparison to Western Blot for HIV-1.

$$\text{Sensitivity} = \frac{\text{True Positives (TP)}}{\text{True Positives (TP)} + \text{False Negatives (FN)}} \times 100$$

$$\text{Specificity} = \frac{\text{True Negatives (TN)}}{\text{True Negatives (TN)} + \text{False Positives (FP)}} \times 100$$

$$\text{Negative Predictive Value} = \frac{\text{True Negative (TN)}}{\text{True Negative (TN)} + \text{False Negative (FN)}} \times 100$$

$$\text{Positive Predictive Value} = \frac{\text{True Positive (TP)}}{\text{True Positive (TP)} + \text{False Positive (FP)}} \times 100$$

IV. Results

Screening Tests

Out of a total of 1282 specimens tested in this country, 1189 were tested on HIVCHEK and 1232 were tested on ELISA(Genetic Systems). The overall sensitivity, specificity, and negative predictive values for HIVCHEK and ELISA is contained in Table (3), while the negative predictive values for blood donors, high risk groups, individuals with symptoms of AIDS, and pregnant women in Zaire is shown in Table (4).

Table (3): Sensitivity, Specificity, and Negative Predictive Values of HIVCHEK and ELISA as Screening Tests for HIV-1 in Zaire. (*Note: As compared to Western Blot)

	HIVCHEK (N = 1189)	ELISA (N = 1232)
True Positive	220	257
True Negative	951	853
False Positive	3	104
False Negative	15	18
Sensitivity	94%	93%
Specificity	99%	89%
Negative Predictive Value	98%	98%

Table (4): Negative Predictive Values for Serum Obtained From Blood Donors, High Risk Groups, Individuals With Symptoms of AIDS, and Pregnant Women Using HIVCHEK and ELISA as Screening Tests for HIV-1 in Zaire.

	Blood Donors	High Risk Groups	Symptoms of AIDS	Pregnant Women
HIVCHEK	98.2% (N=408)	92.3% (N=16)	99.1% (N=642)	97.8% (N=94)
ELISA	98.0% (N=431)	92.3% (N=16)	98.5% (N=663)	97.7% (N=95)

Supplemental Testing

For the agglutination assays, 327 specimens were tested on SERODIA-HIV and 347 were tested on RETROCELL. The overall sensitivity, specificity, and positive predictive values for SERODIA-HIV and RETROCELL is shown in Table (5), while the positive predictive values for blood donors, high risk groups, individuals with symptoms of AIDS, and pregnant women in Zaire is contained in Table 6.

Table (5): Sensitivity, Specificity, and Positive Predictive Values of SERODIA-HIV and RETROCELL as Supplemental Tests for HIV-1 in Zaire.

	SERODIA-HIV (N = 327)	RETROCELL (N = 347)
True Positive	202	221
True Negative	106	95
False Positive	9	16
False Negative	10	15
Sensitivity	95%	94%
Specificity	92%	86%
Positive Predictive Value	96%	93%

Table(6): Positive Predictive Values for Serum Obtained From Blood Donors, High Risk Groups, Individuals With Symptoms of AIDS, and Pregnant Women Using SERODIA-HIV and RETROCELL as Supplemental Tests for HIV-1 in Zaire.

	Blood Donors	High Risk	Symptoms of AIDS	Pregnant Women
SERODIA-HIV	87.5% (N=58)	100% (N=1)	97.3% (N=238)	71.4% (N=17)
RETROCELL	72.7% (N=62)	100% (N=4)	95.1% (N=256)	100% (N=18)

11

Seroprevalence

Table (7): Seroprevalence of HIV-1 in Blood Donors in 5 Sites in Zaire

BLOOD DONORS AT SITE	HIV-1
Kimbanguiste N = 153	8.5%
Kimpese N = 154	10.4%
Vanga N = 77	9.1%
Tshikaji N = 13	23.1%
Karawa N = 42	9.1%

Table (8): Seroprevalence of HIV-1 in 4 Groups in Zaire

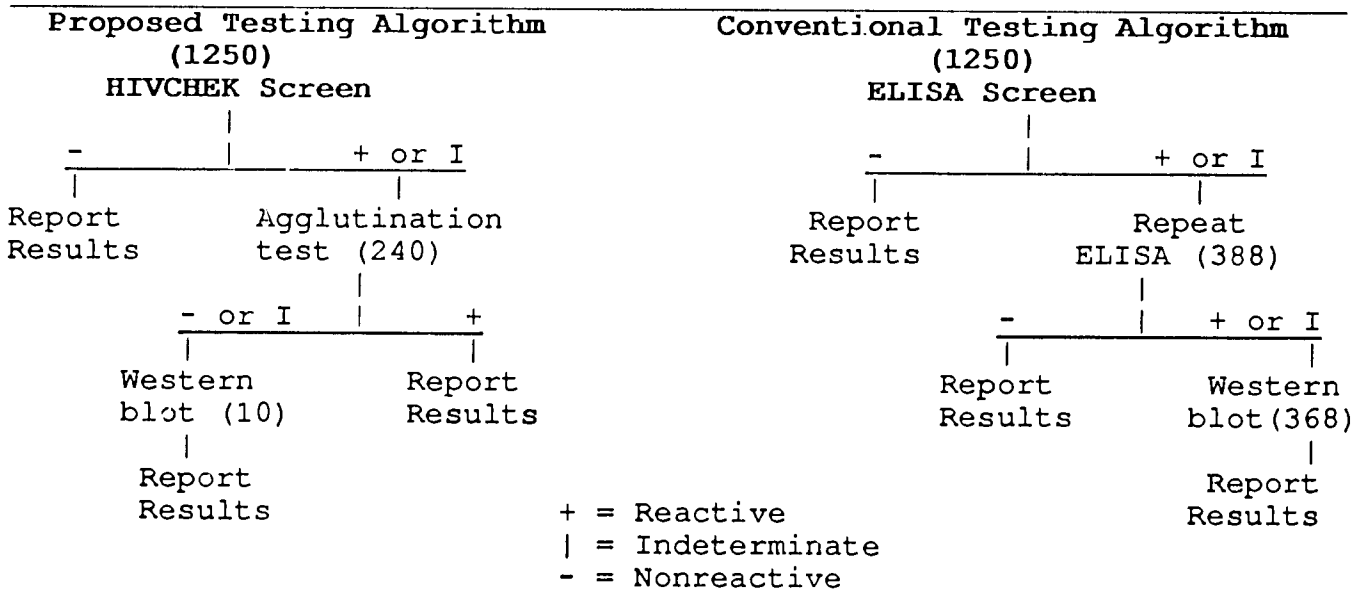
GROUP	HIV-1
Blood Donors N = 439	9.8%
High Risk Groups N = 18	27.8%
Symptoms of AIDS N = 698	31.2%
Pregnant Women N = 99	9.1%

(*Note: The high risk group and symptoms of AIDS group were not clearly defined. Prevalence rates are reported to demonstrate values affecting predictive values.)

Proposed HIV Testing Strategy

The results of a cost comparison of a proposed alternative testing algorithm with that of the conventional strategy using ELISA and Western Blot is presented in Table 9. Savings were demonstrated to be approximately 80% using the proposed testing algorithm.

Table (9): Costs of Proposed Testing Algorithm vs. Conventional Testing Algorithm.



1250 HIVCHEK tests @ $1.50 each = $1,875.00
240 Agglutination tests @ $1.10 each = $264.00
10 Western blot tests @ $40.00 each = $400.00
Total U.S. Dollars = $2,539.00

1638 ELISA tests @ $.90 each = $1,474.20
368 Western blot tests @ $40.00 each = $14,720.00
Total U.S. Dollars = $16,194.20

12

In Table 10, the results obtained for 1,174 specimens tested through the proposed algorithm using HIVCHEK as a screening test and SERODIA-HIV as a supplemental test were compared to their results on the gold standard, Western Blot. Discrepant specimens represented in the center boxes went into the catagory of true negative or true positive depending on the Western Blot result. Specimens for which Western Blot was not done, but were negative by ELISA and HIVCHEK are included in this table.

Table (10): Reliability and predictive values of proposed testing algorithm.

	Western Blot (+)	Western Blot (-)
HIVCHEK (+,I) SERODIA-HIV (+)	200 True Positives	2 False Positives
HIVCHEK (+,I) SERODIA-HIV (-,I)	2 (*True Positives)	4 (*True Negatives)
HIVCHEK (-)	15 False Negatives	951 True Negatives

Sensitivity	93.1%
Specificity	99.8%
Negative Predictive Value	98.5%
Positive Predictive Value	99.0%

V. Discussion

RAPID TESTS

The sensitivity, specificity, and negative predictive values of the HIVCHEK was equal to or better than the ELISA test in all cases. A high negative predictive value is especially important in screening blood because it assures one that blood units that have tested negative are not likely to be infectious. The HIVCHEK had performed well under field conditions as a screening test for antibody to HIV-1.

Agglutination tests, SERODIA-HIV and RETROCELL, were sensitive for detecting antibody to HIV-1 in the group of sera which had been previously screened by HIVCHEK. The specificity was less than desired, but this may have been influenced by the rigid criteria used for Western Blot positivity and the presence of HIV-2 or other related retroviruses in the population.

Each positive predictive value, reported by specimen group, reflected a different HIV prevalence rate. A high positive predictive value is extremely valuable for supplemental tests because it assures one that a positive test result is indicative of HIV infection. While clinical signs and history should also be examined for a more accurate diagnosis, the agglutination tests appeared to offer a valid alternative to Western Blot testing.

From the evaluation of the test kits by the technicians, the RETROCELL was the most difficult for them to interpret results from. HIVCHEK was listed as the easiest test to perform and interpret. SERODIA-HIV and RETROCELL were reported as being equal in the ease of performing the test. In general, they also reported that training in addition to package instructions was not necessary for any of the assays.

TESTING STRATEGY

On the basis of these individual test results and the need for a less expensive and more practical testing strategy than the conventional ELISA and Western Blot, a proposed algorithm was examined as an alternative testing strategy. In the proposed algorithm, blood would first be screened by HIVCHEK. Negative results would be reported immediately, while all positive or indeterminate sera would be given supplemental testing by an agglutination test (SERODIA-HIV or RETROCELL). Specimens that were reactive on both the HIVCHEK and the agglutination test would be reported as reactive for antibody to HIV without further testing. Sera which were positive by HIVCHEK but negative or indeterminate by an agglutination test would be referred for additional testing by Western Blot.

A. COSTS

When contrasting the costs of using the proposed testing algorithm with that of the conventional strategy, there was a demonstrated savings of about 80%. This savings is based on actual test results and actual costs of tests for this study in Zaire and numbers of tests were adjusted to allow for comparison. Costs calculated, however, reflect only the cost of the tests and does not include technician time or any of the other recurrent costs involved in HIV testing. In the proposed algorithm, costs were reduced to 6.37 times less than the conventional strategy using ELISA and Western Blot when applied to 1250 sera with 19% of the sera requiring supplemental testing.

B. RELIABILITY/PRACTICALITY

These cost savings must be considered in conjunction with the reliability and practicality of the testing system. Following the proposed algorithm, there were 2 false positive results out of over 1100 sera tested. These false positive results might be reduced after clinical signs and history of risk behavior were taken into account in diagnosing HIV infection.

In this study, performance of the rapid tests in field laboratories was compared to the current "gold standard" of ELISA and Western Blot tests performed in the reference lab. If the standard testing method were used in field laboratories, reliability of the ELISA tests may be less and sending Western Blot-positive specimens to the reference lab may result in long delays in test reporting. The simple agglutination tests meanwhile can be performed in the field laboratories as supplemental tests and have more immediate test results available to the clinician.

HIV COUNSELING

Rapid, simple tests for HIV which are now commercially available have been shown to be appropriate for use in field situations on the basis of this study. With this new technology, HIV testing in rural hospital settings has become possible, making it necessary to be able to provide counseling services at these testing sites. An additional goal of this project was to prepare guidelines on criteria for pre-/post- test counseling.

A workshop was held to train trainers from the 5 hospitals participating in the study. 19 participants - including facilitators from SANRU and the BCC - met for a period of 5 days.

Topics which were discussed during this workshop included the following:

- clinical criteria for blood transfusion
- organization of donor recruitment
- pre-test counselling
- confidentiality
- reporting of test results
- post-test counselling
- dissemination of HIV/AIDS information within the rural community

A training module for personnel involved with HIV testing in rural hospitals has been developed and was tested during the workshop.

VI. Recommendations

1. Consider using HIVCHEK as a screening assay in laboratories which:

    a) are not equipped with ELISA equipment.
    b) do not support a large volume of testing.
    c) require emergency screening of blood.

2. Consider using the proposed testing algorithm as a cost saving measure to assure sustainability of the HIV testing program.

3. Evaluate new tests in similar field testing conditions as they become available.

4. Implement a counseling program for all testing sites.

5. Initiate a quality assurance program for all testing sites.

VII. References

1. Van de Perre, P., Nzaramba, D., Allen, S., Riggen, C.H., Sprecher-Goldberger, S., Butzler, J.P., Comparison of Six Serological Assays for Human Immunodeficiency Virus Antibody Detection in Developing Countries. J Clin. Microbiol. 26:552-6, 1988.

2. Carlson, J.R., Yee, J.L., Watson-Williams E.J., et al., Rapid, Easy and Economical Screening Test For Antibodies to Human Immunodeficiency Virus. Lancet ii:361-2, 1987.

3. Riggin, C.H., Beltz, R.A., Hung, C., et al., Detection of Antibodies to Human Immunodeficiency Virus by Latex Agglutination With Recombinant Antigen. J Clin. Microbiol. 25:1772-3, 1987.

4. Yoshida, T., Matsui, T., Kobayashi, S., Yamamoto, N., Evaluation of Particle Agglutination Test for Antibody to Human Immunodeficiency Virus. J Clin. Microbiol. 25:1433-7, 1987.

5. Spielberg, F., Mulanga Kabeya, C., Ryder, R.W., et al., Comparative Evaluation of Rapid, Visually Read HIV Antibody Screening Assays at Mama Yemo Hospital, Kinshasa, Zaire. Lancet ii:581-4, 1989.

6. Quinn, T.C., Riggin, C.H., Kline, R.L., et al., Rapid Latex Agglutination Assay Using Recombinant Envelope Polypeptide for the Detection of Antibody to the HIV. JAMA 260(4):510-13, 1988.

7. Chun, P.K., Albert, E.C., Cybulski, R.L., Galvin, J.P., Detection of Antibodies to Human Immunodeficiency Virus (HIV) in Serum and Plasma Using a Novel Immunofiltration Procedure and Collodial Gold. AIDS in Africa Conference Abstract Volume, 1987.