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# HIV SCREENING AND CONFIRMATION: A SIMPLIFIED AND LESS EXPENSIVE TESTING ALGORITHM

by

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*Summary.* — In this study we investigated the performance of fourteen different assays capable of simultaneously detecting antibodies to HIV-1 and HIV-2, referred to as combined screening assays (CSAs), on a panel of 371 sera, with a prevalence of 51.5 % and 1.3 % for HIV-1 and HIV-2 antibodies respectively. The geographic distribution of the sera was as follows; Europe (121), Africa (203) and Latin America (47). These sera were collected from different clinical groups of patients; Asymptomatic (36), AIDS-Related Complex/AIDS patients (18), infected individuals with generalised lymphadenopathy (12), blood donors (149), and subjects with unknown clinical status (156).

The Dupont Western blot (WB) kit for detection of HTLV-III antibodies and the Pasteur new Lav-Blot II kit were used for the confirmation of HIV-1 and HIV-2 infection respectively. Of the 14 tests studied, 9 were enzyme linked immunosorbent assays (ELISAs), and 5 were non-Elisa tests requiring visual reading.

An alternative approach for HIV antibody testing was studied restrospectively, whereby sera positive in an initial CSA (A) were retested on a second CSA (B), that was different from the first. The use of WB was limited to sera that gave discrepant (A+B−) results in the two CSAs. A positive result in both CSAs was reported as anti-HIV positive. A negative result in the first CSA was reported anti-HIV negative. Sensitivity, specificity, cost, and the delta ($\delta$) values (delta values of the ELISA assays) were taken into consideration when selecting suitable pairs of assays.

All the ELISAs scored 100 % sensitivity, but for the non-ELISAs, the sensitivity ranged from 96.0 % to 100 %. The specificity for the ELISAs and non-ELISAs varied from 87.4 % to 100 % and from 51.4 % to 100 % respectively. Delta ($\delta$) values for the ELISAs ranged from 3.82 to 136.68 and from −1.15 to −3.08 for the anti-HIV positive and anti-HIV negative populations respectively.

Of the 121 test combinations studied, 9 (7.4 %) pairs yielded 100 % sensitivity and specificity and 61 (50.4 %) pairs of CSAs required further testing on WB. This implies 100 % positive predictive value, at a cost that was on average 6 times less, and a testing time that was 5 times faster than the conventional algorithm. We conclude that there are several combinations of pairs of CSAs that can be used in the alternative algorithm that can provide accurate results at a much lower cost than the conventional algorithm requiring confirmation by WB of all initially reactive CSA results. There is however a need to further investigate this alternative algorithm and the most appropriate combination of CSAs under real field conditions especially in laboratories with limited resources and less experienced operators.

KEYWORDS: HIV Antibody Assays; Combined HIV-1 and HIV-2 Screening Assays; Alternative Confirmatory Algorithm.

## Introduction

Assays for the simultaneous detection of antibodies to both HIV-1 and HIV-2, referred to as combined screening assays (CSAs), were developed after the human immunodeficiency virus type two (HIV-2) had been discover-

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ed in West Africa (2) and following the observation that enzyme-linked immunosorbent assays (ELISAs) designed for HIV-1 antibody detection were only capable to detect 40 % to 90 % of HIV-2 antibody positive sera (15). The CSAs for HIV-1 and HIV-2 are comparable in accuracy (sensitivity and specificity) to the monospecific HIV-1 or HIV-2 tests (4). However some CSAs show a lower specificity with African sera (13). Therefore, initially reactive CSA results should always be confirmed.

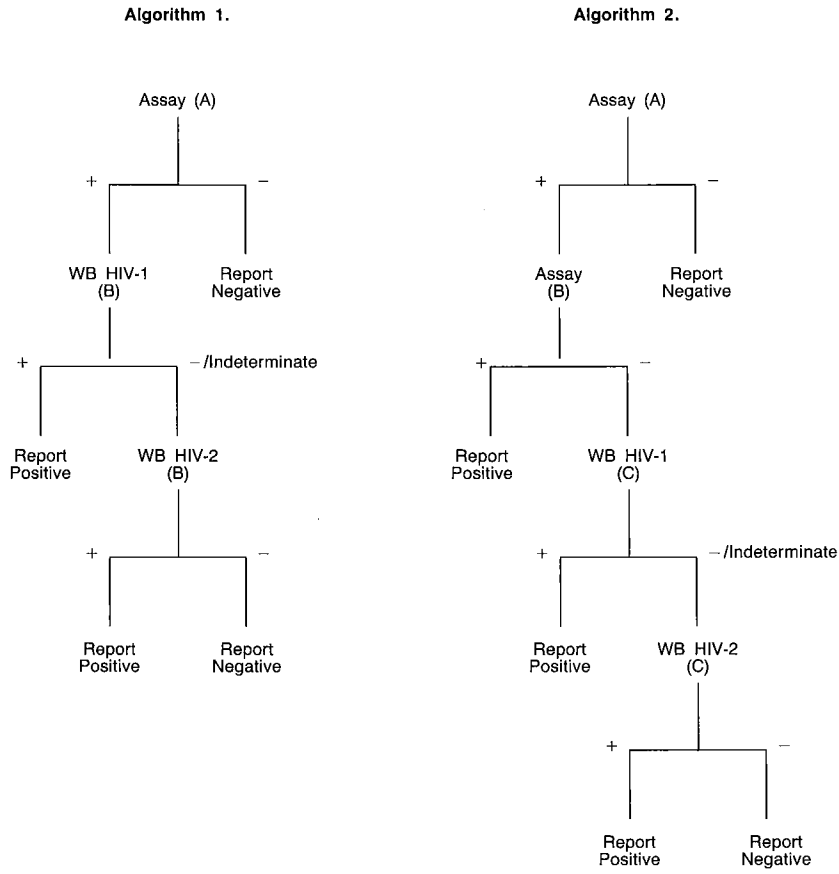


Figure 1
Schematic representation of the conventional (1) and the alternative confirmatory algorithm (2).

In the conventional testing algorithm (Figure 1, algorithm 1) sera reactive in the CSAs are confirmed using the WB. The conventional algorithm may require two WB tests, one for HIV-1 and one for HIV-2 thereby increasing the cost. In addition, WB is time consuming, difficult to interpret, has not yet been standardized and may yield a significant number of indeterminate results (9).

In this paper, the sensitivity, specificity and cost of the conventional testing algorithm were compared to that of an alternative algorithm (Figure 1,

algorithm 2) in which samples, reactive on one CSA were retested on a second CSA and only samples that produced discordant results were tested by the WB.

## Materials and methods

### *Sera*

A panel of 371 human sera presented under code and consisting of 121 sera of European origin, 203 sera of African origin and 47 sera of South American origin was used to evaluate the 14 CSAs. The panel contained 191 (51.5%) HIV-1 antibody positive sera and 5 (1.3%) HIV-2 antibody positive sera. The sera were obtained from different clinical groups of patients: 36 infected asymptomatic subjects, 18 patients with ARC/AIDS, 12 infected persons with generalized lymphadenopathy, 149 blood donors and 156 subjects of unknown clinical status. All samples were frozen in aliquots and thawed at least once and at most twice.

### *HIV antibody assays*

Fourteen commercially available combined screening assays for the simultaneous detection of antibodies to HIV-1 and HIV-2 were used to test all the sera in the panel. All assays were performed according to the instructions provided by the manufacturers. Nine of the assays used the ELISA format and five assays utilized the rapid immunodot format and were visually interpreted independently by three operators. Table 1 summarizes the characteristics of the 14 combined screening assays (CSAs) evaluated. The price per test varied from US$ 0.9 to US$ 4.8, with an average price per test of US$ 2.4.

### *Reference Test*

The DuPont WB kit for detection of HTLV-III antibodies and the Pasteur New Lav-Blot II kit were used for the confirmation of HIV-1 and HIV-2 antibodies respectively. The assays were performed according to the instructions of the manufacturer. A WB HIV-1 or WB HIV-2 result was considered positive when 2 of 3 *env* bands (env precursor, external and transmembrane glycoproteins) with or without *gag* and/or *pol* bands were present (1). A WB result was considered negative when no HIV specific bands were present. When any band pattern not considered positive or negative was observed, the result was considered to be indeterminate.

### *Study design and data analysis*

All sera of the panel were tested with all 14 screening assays and with the DuPont WB kit for confirmation of HIV-1 positivity. Sera, positive in any

of the CSAs, but either negative or indeterminate on the HIV-1 WB were further tested on the Pasteur New Lav-Blot II. The results of the Western blot assays were regarded as final.

TABLE 1
**Characteristics of the combined antibody (HIV-1/HIV-2) assays studied**

| No. | Assay (abreviation) | Manufacturer | Type of assay | Type of antigen | Cost/test[1] (US$) |
|---|---|---|---|---|---|
| A. | *ELISAs* | | | | |
| 1. | Elavia Mixt (EIAEM) | Pasteur | Indirect ELISA | HIV lysate | 2.1 |
| 2. | Recombinant HIV1/HIV2 EIA (EIAA) | Abbott | Indirect ELISA | Recombinant protein | 1.8 |
| 3. | Enzygnost Anti HIV1+2 (EIAE) | Behringwerke | Indirect ELISA | Synthetic peptide | 2.3 |
| 4. | Detect-HIV (EIADT) | Biochem | Indirect ELISA | Synthetic peptide | 2.5 |
| 5. | Biochrom HIV1/2 ELISA Modul-test (EIAB) | Biochrom | Indirect ELISA | Synthetic peptide | 0.9 |
| 6. | DuPont HIV1/HIV2 ELISA (EIAD2) | DuPont | Indirect ELISA | Synthetic peptide/ Recombinant protein | 1.3 |
| 7. | Vironostika HIV Mixt (EIAVM) | Organon Teknika | Indirect ELISA | HIV lysate/Synthetic peptide | 1.8 |
| 8. | Anti-HIV1/HIV2 EIA «Roche» (EIAR) | Hoffmann-LaRoche | Indirect ELISA | Recombinant protein | 1.7 |
| 9. | Wellcozyme HIV1+2 (EIAW2) | Wellcome | Sandwich ELISA | Recombinant protein | 1.5 |
| B. | *Non-ELISAs* | | | | |
| 10. | Testpack HIV1/2 Ab (TESTP) | Abbott | Immunodot | Recombinant protein | 4.8 |
| 11. | HIV Chek 1+2 (CHEK2) | DuPont | Immunodot | Synthetic peptide/ Recombinant protein | 4.0 |
| 12. | Immunocomb Bi-Spot (BISP) | PBS Orgenics | Immunodot | Synthetic peptide | 4.0 |
| 13. | Genie HIV1/HIV2 (GEN2) | Genetic Systems | Immunodot | Synthetic peptide | 3.5 |
| 14. | Recodot (RECO) | Waldheim Pharmazeutika | Immunodot | Recombinant protein | 2.0 |

[1] Costs were provided by the distributor of the test in Belgium during the period of evaluation of the assay (1989-1991). Costs may change, according to the country and the number of tests ordered.

Results obtained from the combinations of different combined screening assays during the alternative algorithm 2 (Figure 1) were then analyzed retrospectively. Only assays with a 100% sensitivity were used as the first assay in the pairings. The test combination analysis was done with the Epi-info 5.0, computer statistical package, CDC, Atlanta.

The sensitivity and specificity of the different assays and different test combinations were calculated taking the Western blot results as the «gold standard». Indeterminate Western blot results were not used in the calculations of sensitivity and specificity. The simple non-ELISA tests requiring visual reading were read independently by three operators. Agreement in two of three readings determined the overall result. The sensitivity and specificity of each CSA was calculated as described in the legend of Table 2. The sensitivity of each test combination was calculated as the number of WB confirmed positive sera obtained with the test combination, divided by the total number of WB confirmed positive sera, multiplied by a hundred.

The specificity of each test combination was calculated as the number of specimens considered negative by the test combination, divided by the total number of WB negative specimens, multiplied by a hundred.

95 % confidence limits (95 % CL) on sensitivity and specificity data were calculated as previously described (14).

TABLE 2

**Sensitivity, specificity and delta values of the different HIV antibody combined screening assays used in the study**

| No. Assay (1) | % sensitivity (2) (+ 95 % CL) (4) | % specificity (3) (+ 95 % CL) | Delta (δ) values (5) | |
|---|---|---|---|---|
| | | | WB− sera | WB+ sera |
| A. *ELISAs* | | | | |
| 1. EIAEM | 100.0 (98.1-100.0) | 93.1 (88.3-96.4) | − 2.23 | 136.68 |
| 2. EIAA | 100.0 (98.1-100.0) | 97.1 (93.4-99.1) | − 1.15 | 3.82 |
| 3. EIAE | 100.0 (98.1-100.0) | 93.7 (89.0-96.8) | − 2.02 | 20.37 |
| 4. EIADT | 100.0 (98.1-100.0) | 97.1 (93.4-99.1) | − 2.41 | 33.74 |
| 5. EIAB | 100.0 (98.1-100.0) | 97.7 (94.2-99.4) | − 2.13 | 10.03 |
| 6. EIAD2 | 100.0 (98.1-100.0) | 87.4 (81.6-92.0) | − 1.24 | 14.06 |
| 7. EIAVM | 100.0 (98.1-100.0) | 100.0 (97.9-100.0) | − 3.08 | 15.59 |
| 8. EIAR | 100.0 (98.1-100.0) | 97.1 (93.4-99.1) | − 2.47 | 17.24 |
| 9. EIAW2 | 100.0 (98.1-100.0) | 94.9 (90.8-97.5) | − 2.20 | 47.48 |
| B. *Non-ELISA* | | | | |
| 10. TESTP | 100.0 (98.1-100.0) | 97.1 (93.4-99.1) | — | — |
| 11. HIV CHEK | 99.0 (96.4-99.9) | 100.0 (97.9-100.0) | — | — |
| 12. BISP | 99.0 (96.4-99.9) | 99.4 (96.9-100.0) | — | — |
| 13. GEN2 | 99.5 (97.2-100.0) | 99.4 (96.9-100.0) | — | — |
| 14. RECO (6) | 96.9 (93.4-99.0) | 51.4 (43.8-59.0) | — | — |

(1) For full names of the assays, see Table 1.
(2) The percentage sensitivity was calculated by dividing the number of Western blot (WB) confirmed positive specimens detected initially by the combined screening assay (HIV-1 + HIV-2) kit under evaluation, by the total number of WB confirmed positives, multiplied by a 100.
(3) The percentage specificity was calculated by dividing the number of WB confirmed negative specimens detected by the combined screening assay (HIV-1 + HIV-2) kit under evaluation, by the total number of WB confirmed negatives, multiplied by a 100.
(4) Figures in parentheses are the 95 % confidence limits, calculated based on reference 14.
(5) Delta (δ) values for the HIV-1/HIV-2 antibody positive and antibody negative samples were calculated by dividing the mean of the $Log_{10}$ sample to cut-off optical density ratio's by the standard deviation of each population.
WB − : Western blot confirmed negative sera.
WB + : Western blot confirmed positive sera.
(6) 56 sera, indeterminate in the Recodot assay, were not retested, according to the instructions of the manufacturer.

Determination of the delta (δ) values permits comparison of the efficacy of ELISA type CSAs to separate the negative and positive HIV antibody serum populations from the cut-off value, as described by Crofts *et al.* (3) and Maskill *et al.* (7). In brief, they were calculated by dividing the mean optical density/cut-off (OD/CO) ratio ($Log_{10}$) by the standard deviation of each population. OD/CO ratio's were calculated by dividing each reading by the relevant cut-off. The optical densities for sera giving readings greater than that able to be measured by the ELISA reader were given an optical density value of 4.000.

The cost of the alternative algorithm (Figure 1, algorithm 2) for each pair of combined screening assays was calculated taking into account the cost of each individual assay and the number of sera tested by each of the assays.

The cost savings ratio was calculated as the cost per serum to test 371 sera by the conventional algorithm (Figure 1, algorithm 1) divided by the cost per serum to test the same number of sera by the alternative algorithm (Figure 1, algorithm 2).

## Results

As shown in Table 2, all the ELISAs showed a sensitivity of 100 %, and a specificity varying from 87.4 % to 100 %. The Vironostika HIV Mixt scored a 100 % sensitivity and specificity. Positive delta values ($\delta+$) ranged from 3.82 to 136.68, while negative delta values ($\delta-$) varied from $-$ 1.15 to $-$ 3.08. For the simple assays with visual reading, only one test, Testpack HIV1/HIV2 Ab, scored a 100 % sensitivity. Specificities of these simple assays varied from 51.4 % to 99.4 %.

A number of false positive sera were detected by all but one of the assays under evaluation. Twenty five sera gave false positive reactions in two different assays, four sera gave false positive reactions in three different assays, one serum was false positive in five different assays, and fifty sera were indeterminate in WB.

Based on the total HIV-1 and HIV-2 seroprevalence (52.8 %) in the sera used in this study, the application of the conventional testing algorithm (where a CSA of an ELISA format is used to screen for HIV-1 and HIV-2 and confirmation of positive results is performed using both the HIV-1 WB and the HIV-2 WB assay) was very expensive. It led to an average cost of US$ 26.7 per sample tested. An average cost of US$ 29.3 per sample tested was obtained if a simple CSA with visual reading was used prior to the WB.

Therefore, in the alternative algorithm, sera found to be positive in the initial combined screening assay were retested using a different combined screening assay. Only sera that gave discrepant screening assay results were tested by WB (Figure 1, algorithm 2). A total of 121 pairs of different CSAs were examined according to algorithm 2. Thirty two of the 72 pairs of ELISA, 34 of the 45 pairs of ELISA with non-ELISA and all 4 pairs of non-ELISA CSAs resulted in 100 % sensitivity and specificity at an average cost that was 6.7, 5 and 3.7 times less than that of the conventional algorithm (Figure 1, algorithm 1). The successful combinations used CSAs with 100 % sensitivity as the first test followed by a CSA which was capable of detecting false positive sera different from those detected by the first CSA.

The cost savings ratio of combinations of CSA pairs which produced 100 % sensitivity and specificity without requiring the use of the Western blot (i.e. no discrepant results were obtained) varied from 5.9 to 11.0 (Table 3). The cost savings ratio of the combinations of CSA pairs which produced 100 % sensitivity and specificity but required the use of the Western blot to resolve the antibody status of discrepant samples ranged from 2.8 to 9.7 (Table 4).

## Discussion

The alternative algorithm which consists of confirming initially reactive CSA specimens by a second and different CSA, and limiting the use of the WB for resolving the anti-HIV status of sera yielding discrepant results led to the correct identification of positive and negative sera in 70 of the possible 121 assay combinations. The average cost of the alternative algorithm was

six times lower (83 % savings) than the price of the conventional algorithm. Mitchell *et al.* (8) using a similar testing algorithm also observed an average cost savings of up to 82 % over the conventional algorithm. These savings were due mainly to the fact that fewer WBs had to be performed in the alternative algorithm. Fewer WB implies a reduced cost and also fewer indeterminate results will be encountered.

TABLE 3

**Comparison of cost savings ratio of the alternative testing algorithm in which different combinations of HIV-1/HIV-2 screening assays produced 100 % sensitivity and specificity without requiring the use of WB**

| No. | Test combination (1) | Cost savings ratio (2) |
|---|---|---|
| A. | *ELISA-ELISA* | |
| 1. | EIAVM-EIAB | 11.0 |
| 2. | EIAVM-EIAD2 | 10.1 |
| 3. | EIAVM-EIAW2 | 9.7 |
| 4. | EIAVM-EIAR | 9.4 |
| 5. | EIAVM-EIAA | 9.0 |
| 6. | EIAVM-EIAE | 8.4 |
| 7. | EIAVM-EIADT | 8.2 |
| 8. | EIAVM-EIAEM | 7.7 |
| B. | *ELISA - non-ELISA* | |
| 9. | EIAVM-TESTP | 5.9 |

(1) For full names of the assays, see Table 1.
(2) The cost savings ratio was calculated as the cost per serum to test 371 sera by the conventional algorithm (Figure 1, algorithm 1) divided by the cost per serum to test the same 371 sera by the alternative algorithm (Figure 1, algorithm 2).

In this study, all ELISAs performed yielded 100 % sensitivity, however, among the simple non-ELISA assays only Testpack HIV1/2 Ab showed 100 % sensitivity, and was therefore the only non-ELISA assay used as a first assay in a pairing.

The delta values for the ELISAs should be considered when pairing two ELISA CSAs with the same sensitivity and specificity. Assays having the higher positive ($\delta+$) and negative ($\delta-$) delta values are expected to obtain a clearer separation from the cut off value of the anti-HIV positive and anti-HIV negative specimens. By combining a first assay having a high positive delta ($\delta+$) value with a second assay having a high negative delta ($\delta-$) value, one can expect to select a combination of tests that is capable of tolerating a large margin for variation of test results without the occurrence of false negative or false positive results (7). Combinations of two ELISAs in algorithm 2 using as the first test an ELISA with the highest $\delta+$ value (EIAEM $\delta+$ 136.68), followed by the ELISA with the highest $\delta-$ value (EIAVM $\delta-$ 3.08), resulted in no false positive or false negative results. However, the combination of two ELISAs using as first test the ELISA with the lowest $\delta+$ value (EIAA $\delta+$ 3.82), followed by one of the ELISAs with the lowest $\delta-$ value (EIAD2 $\delta-$ 1.24) resulted in four false positive results and a specificity for the algorithm of only 88.2 %.

TABLE 4

**Comparison of cost savings ratio of the alternative testing algorithm in which different combinations of HIV-1/HIV-2 screening assays produced 100 % sensitivity and specificity requiring the use of WB to confirm discrepant results**

| No. | Test combination (1) | No. WB (2) | Cost savings ratio |
|---|---|---|---|
| A. | *ELISA - ELISA* | | |
| 1. | EIAB-EIAVM | 4 | 9.7 |
| 2. | EIAB-EIAE | 4 | 8.7 |
| 3. | EIAR-EIAW2 | 5 | 7.5 |
| 4. | EIAR-EIAVM | 5 | 7.3 |
| 5. | EIAA-EIAVM | 5 | 7.1 |
| 6. | EIAA-EIAE | 5 | 6.6 |
| 7. | EIAW2-EIAR | 9 | 6.5 |
| 8. | EIAW2-EIAVM | 9 | 6.4 |
| 9. | EIADT-EIAVM | 5 | 6.1 |
| 10. | EIADT-EIAR | 5 | 6.1 |
| 11. | EIADT-EIAE | 5 | 5.8 |
| 12. | EIAE-EIAB | 11 | 5.7 |
| 13. | EIAB-EIAEM | 4 | 5.7 |
| 14. | EIAE-EIAD2 | 11 | 5.5 |
| 15. | EIADT-EIAEM | 5 | 5.4 |
| 16. | EIAEM-EIAB | 12 | 5.3 |
| 17. | EIAE-EIAA | 11 | 5.2 |
| 18. | EIAEM-EIAVM | 12 | 4.9 |
| 19. | EIAE-EIADT | 11 | 4.8 |
| 20. | EIAEM-EIADT | 12 | 4.6 |
| 21. | EIAD2-EIAVM | 22 | 4.5 |
| 22. | EIAD2-EIAE | 22 | 4.3 |
| 23. | EIAR-EIADT | 5 | 3.7 |
| 24. | EIAE-EIAVM | 11 | 2.8 |
| B. | *ELISA - non-ELISA* | | |
| 25. | EIAB-GEN2 | 5 | 8.1 |
| 26. | EIAVM-RECO | 6 | 7.0 |
| 27. | EIAVM-GEN2 | 1 | 6.7 |
| 28. | EIAB-BISP | 6 | 6.1 |
| 29. | EIAB-CHEK2 | 6 | 6.1 |
| 30. | EIAB-TESTP | 4 | 6.0 |
| 31. | EIAVM-BISP | 2 | 6.0 |
| 32. | EIAVM-CHEK2 | 2 | 6.0 |
| 33. | EIAA-RECO | 11 | 5.8 |
| 34. | EIAR-GEN2 | 6 | 5.6 |
| 35. | EIAA-GEN2 | 6 | 5.5 |
| 36. | EIADT-RECO | 11 | 5.2 |
| 37. | EIAR-BISP | 7 | 5.1 |
| 38. | EIAA-CHEK2 | 7 | 5.1 |
| 39. | EIAR-CHEK2 | 7 | 5.1 |
| 40. | EIAA-BISP | 7 | 5.1 |
| 41. | EIADT-GEN2 | 6 | 4.9 |
| 42. | EIAW2-CHEK2 | 11 | 4.7 |
| 43. | EIAW2-BISP | 11 | 4.7 |
| 44. | EIADT-BISP | 7 | 4.6 |
| 45. | EIADT-CHEK2 | 7 | 4.6 |
| 46. | EIADT-TESTP | 5 | 4.5 |
| 47. | EIAE-GEN2 | 12 | 4.3 |
| 48. | EIAEM-GEN2 | 12 | 4.2 |
| 49. | EIAE-BISP | 13 | 4.1 |
| 50. | EIAE-CHEK2 | 13 | 4.1 |
| 51. | EIAEM-BISP | 13 | 4.0 |
| 52. | EIAEM-CHEK2 | 13 | 4.0 |
| 53. | EIAE-TESTP | 11 | 4.0 |
| 54. | EIAD2-GEN2 | 23 | 3.8 |
| 55. | EIAD2-CHEK2 | 24 | 3.6 |
| 56. | EIAD2-BISP | 24 | 3.6 |
| 57. | EIAW2-TESTP | 9 | 3.3 |
| C. | *non-ELISA - non-ELISA* | | |
| 58. | TESTP-RECO | 11 | 3.9 |
| 59. | TESTP-GEN2 | 6 | 3.8 |
| 60. | TESTP-CHEK2 | 7 | 3.6 |
| 61. | TESTP-BISP | 7 | 3.6 |

(1) For full names of the assays, see Table 1.
(2) Number of sera confirmed by Western blot that were reactive by screening assay A but non-reactive by screening assay B.

No systematic correlation between $\delta$ values of CSAs utilizing the ELISA format and the cost and accuracy of algorithm 2 was observed. It is clear that factors other than just the $\delta$ values are important when selecting a suitable pair of CSAs for use in algorithm 2. The small sample size of our panel did not make it possible though to confirm this hypothesis.

Of the 121 test combinations studied, 9 (7.4 %) pairs not requiring further testing on WB yielded 100 % sensitivity and specificity (Table 3), and 61 (50.4 %) pairs of CSAs (Table 4) gave discordant results, thus requiring further testing on WB. This implies a 100 % positive predictive value which is important because it assures that a positive test result reported by algorithm 2 was indicative of HIV infection. It is critical that the first assay in the combination has a sensitivity approximating 100 %, since this determines the overall sensitivity of algorithm 2 (Figure 1). When pairs of assays recognizing the same false positive specimens are used in algorithm 2, false positive results will occur because such sera will not be tested by WB. The specificity of the test combination in algorithm 2 can be improved by combining assays that do not detect the same false positive specimens. This is generally achieved by combining assays that have different test principles (e.g. indirect or competitive) and different types of antigen preparations (e.g. synthetic peptides, recombinant proteins).

Since all the 14 CSAs were tested on the same panel of sera, assay combinations which were not capable of yielding a 100% specificity could be readily identified.

The choice of a combination of ELISA or non-ELISA assays in a given situation depends on the number of specimens to be tested, the inter-reader variability of the non-ELISA assays, and the laboratory equipment available. In this study, we demonstrated that even combinations of two simple assays could result in 100% sensitivity and specificity. These combinations could be performed in a less sophisticated laboratory, giving a result in a relatively short time. Unfortunately these simple non-ELISA assays are still expensive for use in strictly resource-limited settings. The mean cost per test for the ELISAs and simple non-ELISA assays was US$ 1.8 and US$ 3.7 respectively.

The order in which two assays are combined is important. This is evident when comparing test combination 2 of Table 3, in which none of the sera was initially false positive, with test combination 21 of Table 4. In the latter, using a first assay which is 100% sensitive but with a low specificity, 22 initially false positive sera had to be retested, thereby reducing the cost savings ratio of the alternative algorithm considerably.

In order to get an idea of the real savings involved, we calculated the cost-savings for the two algorithms (with the highest and lowest cost-savings ratios (algorithms 1 and 24)) (Table 4), under two different prevalence rates (1% and 10%). Using algorithm one 10,000 times in a population with 1% seroprevalence leads to a costing-savings ratio of 1.3, this is a net gain of US$ 3,800 as compared to the conventional testing strategy. When the seroprevalence rises to 10%, the cost-savings ratio is 2.6 and the gain increases up to US$ 41,800. Applying algorithm 24, which has the lowest cost-savings ratio, we have a ratio of 1.0 and 1.2 respectively, this is a net gain of US$ 2,911 and US$ 41,232 for a population with 1% and 10% seroprevalence respectively.

This study shows that there are several combinations of CSAs, that can be used in the alternative algorithm which are capable of providing as accurate results, at a lower cost as the conventional algorithm requiring the use of WB for confirmation of initially reactive CSA results. The two algorithms applied in this study did not use repeatably reactive results. Van der Groen *et al.* (12) have shown that there was no gain in accuracy when using testing algorithms in which initially reactive samples were retested with the same assay. However, gains in accuracy were obtained when initially reactive samples were tested only once with a different second or third screening assay.

The data from this study were obtained from the retrospective analysis of results, produced from the testing of a panel of sera with 14 different CSAs. However, the extent to which these data can be extrapolated to actual field conditions is not known. It has been shown that the performance of screening assays varies greatly with testing laboratory conditions (5, 6, 8, 10, 11). Staff training and appropriate quality control procedures may improve the performance of the tests and consequently the testing algorithms in laboratories with limited resources and less experienced operators. Therefore studies will be required in developing countries to determine which assays are most appropriate for pairing in the alternative algorithm described here. The assays

and algorithm should be evaluated on a panel of regionally collected sera which reflects the prevailing anti-HIV seroprevalence. In such studies, the sensitivity, specificity, and delta values for the different assays should be used in selecting the appropriate combination of assays.

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**Diagnostic et confirmation de l'infection au VIH : un algorithme de dépistage simplifié et moins coûteux.**

*Résumé.* — Dans cette étude, nous avons évalué quatorze tests différents, capables de détecter simultanément des anticorps anti-VIH1 et anti-VIH2, et auxquels on se réfère comme tests de dépistage combinés, sur une série de 371 sérums. Les prévalences des anticorps anti-VIH1 et anti-VIH2 étaient respectivement de 51,5 % et de 1,3 %. La répartition géographique de ces sérums était la suivante : 121 sérums d'Europe, 203 sérums d'Afrique, 47 sérums d'Amérique latine. Ces sérums ont été collectés chez des patients appartenant à différents groupes cliniques : 36 patients asymptomatiques, 18 patients Sidéens ou ARC, 42 patients infectés avec une lymphadénopathie généralisée, 156 donneurs de sang et 119 patients dont le stade clinique n'a pu être déterminé.

Le Western blot (WB) Dupont pour la détection d'anticorps anti-HTLV-III et la trousse New Lav Blot II de Pasteur ont été utilisés pour la confirmation des infections à VIH-1 et à VIH-2 respectives. Neuf des quatorze tests étudiés étaient des Elisa (Enzyme Linked Immunosorbent Assay), et cinq étaient des tests non-Elisa lus à l'œil nu.

Une méthode alternative pour le dépistage d'anticorps anti-VIH a été évaluée de manière rétrospective en réexaminant dans un test B tous les sérums qui étaient positifs pour le test A, les tests A et B étant des tests de dépistage combinés, différents l'un de l'autre. L'usage du WB a été réservé aux sérums qui donnaient des résultats contradictoires (A + B −) dans les deux tests. Un résultat positif dans les deux tests de dépistage était rapporté comme positif. Un résultat négatif dans le premier test était rapporté comme négatif. La sensibilité, la spécificité, le coût, et les valeurs delta ($\delta$) (quand on couplait un test utilisant la méthode Elisa) étaient considérés en sélectionnant des paires de tests appropriées.

Tous les Elisa avaient une sensibilité de 100 %, tandis que pour les non-Elisa, la sensibilité allait de 96,0 % à 100 %. La spécificité pour les Elisa et les non-Elisa variait respectivement de 87,4 % à 100 % et de 51,4 % à 100 %. Les valeurs delta ($\delta$) pour les Elisa allaient de 3,82 à 136,68 et de − 1,15 à − 3,08 pour les populations anti-VIH positives et négatives respectives.

Neuf paires de tests de dépistage combinés donnaient une sensibilité et une spécificité de 100 % et par conséquent une valeur prédictive positive de 100 %, avec un coût six fois moins élevé, et un temps d'exécution cinq fois plus rapide qu'en utilisant l'algorithme conventionnel.

Notre conclusion est que plusieurs combinaisons de paires de tests de dépistage combinés pourraient être utilisées dans des algorithmes alternatifs, en donnant des résultats aussi exacts, et à un coût nettement inférieur que l'algorithme conventionnel qui nécessite la confirmation par WB de tous les résultats des tests de dépistage combinés initialement réactifs. Cependant, cet algorithme alternatif mérite davantage d'évaluations, particulièrement une combinaison de tests de dépistage combinés applicable à des laboratoires aux ressources limitées et au personnel peu expérimenté.

**Diagnose en bevestiging van HIV infektie : een vereenvoudigd en goedkoper opsporings-algoritme.**

*Samenvatting.* — In deze studie werd op een groep van 371 sera, een totaal van 14 verschillende testen geëvalueerd. Elke test was in staat zowel antilichamen tegen HIV-1 als tegen HIV-2 te ontdekken (gekombineerde opsporingstesten (GOT)). De prevalentie van antilichamen tegen HIV-1 en HIV-2 was respectievelijk 51,5 % en 1,3 %.

Van de 371 sera waren er 121 afkomstig uit Europa, 203 uit Afrika en 47 uit Latijns Amerika. De sera kunnen in de volgende groepen onderverdeeld worden : asymptomatische (36), AIDS/ARC patiënten (18), patiënten met algemene lymphadenopathie (12), bloed donoren (149) en personen met ongekende klinische toestand (156). De Dupont Western blot (WB) test voor het opsporen van HTLV-III antilichamen en de Pasteur New Lav Blot II werden gebruikt respektievelijk ter bevestiging van HIV-1 en HIV-2 infekties.

Bij de 14 onderzochte testen waren er 9 ELISA (Enzyme Linked ImmunoSorbent Assay) en 5 non-ELISA met visuele resultaataflezing.

Retrospektief werd tevens een alternatieve aanpak van HIV antilichaam testing onderzocht. Hiervoor werden sera die positief bevonden werden in een eerste GOT (A) hertest in een andere GOT (B). Het gebruik van WB werd beperkt tot die sera die in beide GOT een ander resultaat

gaven (A+B−). Een positief resultaat in beide GOT werd automatisch aanvaard als anti-HIV positief. Een negatief resultaat in de eerste GOT werd aanvaard als anti-HIV negatief. Sensitiviteit en specificiteit, kostprijs en delta ($\delta$) waarden van de ELISA werden gebruikt bij de selektie van geschikte testparen.

Alle ELISA hadden 100% sensitiviteit, bij de non-ELISA varieerde de sensitiviteit van 96,0% tot 100%. De specificiteit van de ELISA en non-ELISA varieerde respektievelijk van 87,4% tot 100% en van 51,4% tot 100%. De delta ($\delta$) waarden van de ELISA testen varieerden van 3,82 tot 136,68 voor de anti-HIV positieve sera en van − 1,15 tot − 3,08 voor de anti-HIV negatieve sera. Van de 121 bestudeerde testkombinaties waren er 9 (7,4%) paar die zowel 100% specificiteit als 100% sensitiviteit opleverden en 61 (50,4%) paar die een bevestigende WB test vereisten. Dit betekent dat een 100% positieve voorspellende waarde (positive predictive value) bereikt werd tegen een kostprijs die gemiddeld zes maal lager lag en tegen een testduur die gemiddeld vijf maal sneller was dan het gangbare (konventionele) algoritme.

We besluiten dan ook dat er verschillende GOT paren zijn die, gebruikt als alternatieve opstelling, sneller en veel goedkoper tot juiste resultaten leiden dan het konventionele algoritme dat WB bevestiging voorschrijft voor alle initieel positieve resultaten. De nood blijft echter bestaan om dit alternatief algoritme en de meest aangepaste GOT in reële situaties in laboratoria met beperkte middelen en relatief weinig ervaren personeel, uit te testen.

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