Simplified and less expensive confirmatory HIV testing*

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The conventional approach to human immunodeficiency virus (HIV) antibody testing, which relies on confirmation of all initially positive screening results using a Western blot assay, is expensive. In an alternative approach, we retested sera that were positive in an initial screening assay using a second screening assay, which differed from the first, and limited the use of Western blot to those sera that gave discrepant results in the two screening assays. This resulted in 100% sensitivity and specificity at a cost that was, on average, 6.1 times less than that of the conventional approach. This level of sensitivity and specificity was also achieved at a cost that was 9.0 times less than the conventional approach if the Western blot was replaced by a third screening assay that differed from the previous two.

Retesting positive sera using the same assay did not increase the accuracy of the results obtained by testing the sera only once.

Introduction

Enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to human immunodeficiency virus (HIV) were first introduced in 1985. At present, most commercially available ELISAs for HIV have excellent sensitivity and specificity. If we assume a sensitivity and specificity of 99.9%, the proportion of false positives obtained with such ELISAs increases from less than 10% to over 70% as the prevalence of HIV in the population tested decreases from 1.0% to 0.04% (*1*). Therefore, initially positive ELISA results should always be confirmed. In the conventional testing strategy, a positive ELISA screening test is followed by confirmation using Western blot assay. However, this approach has several weaknesses (*2–4*): Western blot is costly and time-consuming, needs sophisticated equipment, is difficult to interpret and standardize, and can yield indeterminate results.

To reduce the number of Western blots required in the conventional approach, we have evaluated the use of pairs of HIV-antibody screening assays, with an emphasis on the more recently developed simple, non-ELISA assays (*5*, *6*) and second-generation assays that employ recombinant HIV proteins or synthetic peptides (*7*, *8*).

The use of this alternative approach could lead to accurate, cheaper, more rapid and/or less equipment-reliant testing for HIV infection.

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Materials and methods

Sera. A panel of 361 human sera (220 of which were of African, 131 of European, and 10 of South American origin) was used. A total of 164 (45%) of the sera were HIV-1 antibody positive. All samples were stored in aliquots and thawed at least once, but at most four times.

Reference test. The results obtained using the various assays and combinations of assays were compared with those obtained using HIV-1 Western blot (Du Pont). A Western blot result was considered positive when at least the bands representative of both HIV-specific *core* and *env* proteins were present.

HIV antibody assays. The majority of the commercially available HIV assays shown in Table 1 were performed on all sera in the panel, according to the instructions provided by the manufacturers; some assays were, however, performed on a smaller number of sera, because the panel was incomplete when they were tested. For the HIV Chek assay, all sera were pretreated with Lipoclean (Behring) to facilitate penetration of the membrane.

Data analysis. Initially all the assays were performed on all sera of the panel; the results that would have been obtained for combinations of pairs of assays using the alternative algorithms (Fig. 1) were then analysed retrospectively.

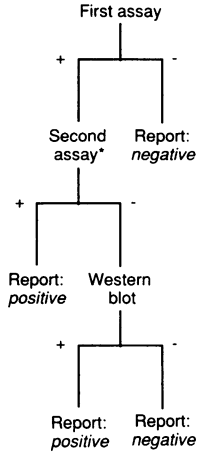
The sensitivity and specificity of the different assays and assay combinations were calculated taking the Western blot results as the standard. The sensitivity of the individual assays was calculated using the initial assay results, and the specificity

Table 1: **Characteristics of the HIV antibody assays studied**

Assay (abbreviation)	Manufacturer	Type of antigen	Type of assay	Cost per test (US$)[a]
Du Pont HIV-1 Rec ELISA (EIA D)	Du Pont	Recombinant proteins	Indirect ELISA	0.9
Recombinant HIV-1 ELISA (EIA A)	Abbott	Recombinant proteins	Indirect ELISA	3.3
Vironostika anti-HTLV-III (EIA V)	Organon Teknika	HIV lysate	Indirect ELISA	2.0
Enzygnost Anti-HIV Micro (EIA B)	Behring	HIV lysate	Competitive ELISA	1.8
Wellcozyme anti-HTLV-III (EIA W)	Wellcome	HIV lysate	Competititve ELISA	1.0
Recombigen HIV-LA (LA CB)	Cambridge BioScience	Recombinant proteins	Agglutination assay	3.0
Serodia-HIV (AG FU)	Fujirebio	HIV lysate	Agglutination assay	1.1
HIV Chek (CHEK)	Du Pont	Recombinant proteins	Immunodot assay	2.5
Immunocomb (ICOMB)	PBS Orgenics	Synthetic peptides	Immunodot assay	2.5
HIV-1 Western blot (WB)	Du Pont	HIV lysate	Immunoblot assay	44.5

[a] Costs were provided by the distributor of the test in Belgium during the period of testing (July 1988–January 1989). Costs may vary according to country and the number of test kits ordered.

Fig. 1. **Schematic representation of algorithm 3: alternative confirmatory strategy using initially positive test results (* = different assay from the first).** Algorithm 4 = algorithm 3, except that repeatedly positive sera in the first and second assays were used; algorithm 5 = algorithm 3, except that the third assay differed from the first and second; and algorithm 6 = algorithm 5, except that the repeatedly positive sera in the first, second and third assays were used.



using the results obtained after initially positive sera had been retested.

Results

The sensitivity and specificity of each of the HIV antibody assays are shown in Table 2. The results indicate that pretreatment of the sera used in the HIV Chek assay with Lipoclean seems to have reduced its sensitivity.

Use of ELISA to screen sera for HIV and confirmation of positive results using Western blot is expensive: on average, US$ 19.7 per test if each serum is screened only once (algorithm 1) and US$ 21.5 per test if positive sera are retested using the screening assay before confirmatory testing (algorithm 2) (Table 3).

In an alternative approach, sera found to be positive in the initial screening assay were retested using a different screening assay, followed by a Western blot only on those sera that gave discrepant screening assays (algorithms 3 and 4, Fig. 1); this resulted in 100% sensitivity and specificity at a cost that was, on average, 6.1 times and 4.7 times less, respectively, than that of the conventional algorithms 1 and 2 (Table 4).

Table 2: **Sensitivity and specificity of the HIV antibody screening assays used in the study**

Assay	No. of sera	Sensitivity (%)[a]	Specificity (%)[b]
Du Pont HIV-1 Rec ELISA	361	100 (98.0–100)[c]	91 (86.0–94.5)
Abbott Recombinant HIV-1 ELISA	361	100 (97.8–100)	98 (95.2–99.6)
Enzygnost Anti-HIV Micro	361	100 (98.0–100)	100 (98.0–100)
Vironostika anti-HTLV-III	361	100 (97.8–100)	99 (96.9–100)
Wellcozyme anti-HTLV-III	361	100 (97.8–100)	100 (98.0–100)
Recombigen HIV-LA	314	95 (93.0–98.2)	96 (93.0–98.2)
Serodia-HIV	351	100 (98.0–100)	97 (93.0–99.0)
HIV Chek	361	95 (90.0–94.5)	99 (96.0–100)
Immunocomb	361	99 (96.0–99.9)	99 (96.0–100)

[a] The % sensitivity was determined by dividing the number of sera that were positive by Western blot, and which were initially positive in the HIV antibody test, by the total number of sera that were confirmed positive by Western blot, and multiplying this by 100.
[b] The % specificity was determined by dividing the number of sera that were confirmed negative by Western blot, and which were negative in the HIV antibody test, by the total number of sera confirmed negative by Western blot, and multiplying this by 100. Initially false-positive sera that twice produced a negative result after repeat testing were included in the calculation.
[c] Figures in parentheses are the 95% confidence limits.

These levels of sensitivity and specificity for algorithms 3 and 4 were also achieved when instead of the Western blot a third screening assay that differed from the previous two was used (algorithms 5 and 6, Fig. 1); in this case the cost was, on average, 9.0 times and 6.9 times lower, respectively, than that of the conventional algorithms 1 and 2 (Table 5).

Not only combinations of three different ELISAs (Table 5, No. 53, 54), and of ELISAs with simple, non-ELISA tests (Table 5, No. 43–52), but also combinations of three different simple, non-ELISA tests (Table 5, No. 37–42) resulted in 100% sensitivity and specificity. These combinations cost, on average, 7.8 and 5.0 times less than the conventional algorithms 1 and 2. The cost per test was US$ 2.0–2.4 with algorithm 5 (Table 5, No. 38, 40, 42).

Although a relatively small number of sera were tested, the test combinations in Tables 4 and 5 had 100% sensitivity and specificity, with the exception of the combination Immunocomb + HIV Chek (Table 4), whose sensitivity was only 98.8%. This arose because the sensitivity of Immunocomb, the first assay in the combination, was 98.8%.

Discussion

When a combination of two or three HIV screening assays is employed, and use of the expensive Western

Table 3: **Cost per test for HIV antibody screening using the conventional algorithms 1 and 2, with Western blot to confirm all positive screening results (see text)[a]**

No.	Algorithm	Test combination[b] 1st	Test combination[b] 2nd	No. of sera tested[c]	No. of sera to be confirmed by Western blot	Cost per test (US$)[d]
1	1	AG FU	WB	189	61	15.5
2	2	AG FU	WB	189	61	15.9
3	1	ICOMB	WB	189	61	17.6
4	2	ICOMB	WB	189	61	17.7
5	1	EIA B	WB	361	164	22.0
6	2	EIA B	WB	361	164	22.8
7	1	EIA D	WB	361	183	23.5
8	2	EIA D	WB	361	177	23.2
9	2	EIA W	WB	361	164	21.7
10	2	EIA V	WB	361	168	23.6
11	2	EIA A	WB	361	168	25.5

[a] Algorithm 1: Western blot confirmation of initially positive ELISA or non-ELISA screening results; algorithm 2: Western blot confirmation of repeatedly reactive ELISA or non-ELISA screening results.
[b] See Table 1 for abbreviations.
[c] The first assays were evaluated using a panel of 189 sera with a 32% HIV-1 antibody prevalence. The serum panel was later extended to 361 sera with a 45% HIV-1 antibody prevalence.
[d] The cost per test was calculated for each combination, taking into account the cost of each assay (see Table 1) and the number of sera tested using each assay.

Table 4: **Comparison of the sensitivity, specificity, and cost per test for HIV antibody screening, using the alternative algorithms 3 and 4 with pairs of ELISA or non-ELISA screening assays in combination with Western blot[a]**

No.	Algorithm	Test combination[b] 1st	2nd	3rd	No. of sera tested	No. of sera to be confirmed[c]	Sensitivity[d] (%)	Specificity[e] (%)	Cost per test (US$)[f]	Price ratio[g]
12	3	AG FU	ICOMB	WB	189	2	100	100	2.4	6.5
13	4	AG FU	ICOMB	WB	189	2	100	100	3.6	4.4
14	3	AG FU	LA CB	WB	103	2	100	100	3.2	5.9
15	4	AG FU	LA CB	WB	103	2	100	100	4.9	3.9
16	3	AG FU	CHEK	WB	189	5	100	100	3.1	5.0
17	4	AG FU	CHEK	WB	189	5	100	100	5.7	2.8
18	3	ICOMB	AG FU	WB	189	5	100	100	4.1	4.3
19	4	ICOMB	AG FU	WB	189	2	100	100	4.5	3.9
20	3	ICOMB	LA CB	WB	126	1	100	100	3.9	4.6
21	4	ICOMB	LA CB	WB	126	0	100	100	5.5	3.4
22	3	ICOMB	CHEK	WB	361	9	98.8	100	4.8	4.8
23	4	ICOMB	CHEK	WB	361	8	98.8	100	6.9	3.5
24	3	AG FU	EIA B	WB	189	2	100	100	2.2	7.0
25	4	AG FU	EIA B	WB	189	2	100	100	3.1	5.1
26	4	AG FU	EIA W	WB	189	2	100	100	2.6	6.1
27	3	AG FU	EIA D	WB	189	2	100	100	1.9	8.2
28	4	AG FU	EIA D	WB	189	2	100	100	2.6	6.1
29	3	EIA B	EIA D	WB	361	0	100	100	2.5	8.8
30	4	EIA B	EIA D	WB	361	0	100	100	3.5	6.5
31	3	EIA D	EIA B	WB	361	19	100	100	4.2	5.6
32	4	EIA D	EIA B	WB	361	13	100	100	4.7	4.9
33	4	EIA D	EIA W	WB	361	13	100	100	3.9	6.0
34	4	EIA W	EIA D	WB	361	0	100	100	2.3	9.4
35	4	EIA V	EIA A	WB	361	4	100	100	6.5	3.6
36	4	EIA A	EIA V	WB	361	4	100	100	7.2	3.5

[a] Algorithms 3 and 4 are depicted in Fig. 1.
[b] See Table 1 for abbreviations.
[c] No. of sera with discrepant results in the 1st and 2nd assay to be confirmed by Western blot.
[d] The % sensitivity was obtained by dividing the number of Western-blot-confirmed positive sera in the test combination by the total number of Western-blot-confirmed positive sera, and multiplying this by 100.
[e] The % specificity was obtained by dividing the number of Western-blot-confirmed negative sera obtained with the test combination by the total number of Western-blot-confirmed negative sera, and multiplying this by 100
[f] See footnote *d*, Table 3.
[g] The cost per serum to test *n* sera with the conventional algorithms 1 or 2, divided by the cost per serum to test the same number of sera with the alternative algorithms 3 or 4, respectively.

blot is limited only to discrepant results or omitted entirely, it is critical that the first assay in the combination be the more sensitive, since this is the factor that limits the sensitivity of the combination.

The specificity of the test combinations can be improved by combining assays that have a different format, i.e., a different type of assay and/or type of antigen. It is then less likely that a nonspecific effect in one assay will interfere also in the other (*8*).

The data obtained using algorithms 2, 4, and 6 suggest that the accuracy of the test combinations does not improve by retesting initially positive sera using the same assay. Equally accurate results were obtained, at lower cost, when each serum was tested only once in each screening assay, as indicated by the findings obtained using algorithms 1, 3, and 5.

It might be thought that the best test combination would be the one that can detect all true positive and negative sera at the lowest cost. However, the cost of a test or test combination is clearly not the only factor that plays a role in the selection; the operational characteristics of the assays, as well as the HIV antibody prevalence in the population studied, might also be decisive.

Especially for small blood transfusion centres in developing countries, where only a few sera are screened, it is important that a combination of less sophisticated, non-ELISA assays can detect all true HIV positive and negative sera.

Our findings on alternative less expensive confirmatory strategies for HIV antibody screening should be followed up by further evaluations of

Table 5: **Comparison of the sensitivity, specificity and cost per test for HIV antibody screening, using combinations of three different assays, without Western blot**[a]

No.	Algorithm	Test combination[b] 1st	2nd	3rd	No. of sera tested	No. of sera to be confirmed[c]	Sensitivity (%)[d]	Specificity (%)[d]	Cost per test (US$)[e]	Price ratio[f]
37	6	AG FU	ICOMB	LA CB	189	2	100	100	3.1	5.1
38	5	AG FU	ICOMB	LA CB	189	2	100	100	2.0	7.8
39	6	AG FU	ICOMB	CHEK	189	2	100	100	3.1	5.1
40	5	AG FU	ICOMB	CHEK	189	2	100	100	2.0	7.8
41	6	AG FU	LA CB	ICOMB	103	2	100	100	4.1	4.7
42	5	AG FU	LA CB	ICOMB	103	2	100	100	2.4	7.9
43	6	AG FU	EIA B	ICOMB	189	2	100	100	2.7	5.9
44	5	AG FU	EIA B	ICOMB	189	2	100	100	1.8	8.6
45	6	AG FU	EIA W	ICOMB	189	2	100	100	2.2	7.2
46	6	AG FU	EIA D	ICOMB	189	2	100	100	2.1	7.6
47	5	AG FU	EIA D	ICOMB	189	2	100	100	1.4	11.1
48	6	AG FU	EIA B	LA CB	189	2	100	100	2.7	5.9
49	5	AG FU	EIA B	LA CB	189	2	100	100	1.8	8.6
50	6	AG FU	EIA W	LA CB	189	2	100	100	2.2	7.2
51	6	AG FU	EIA D	LA CB	189	2	100	100	2.1	7.6
52	5	AG FU	EIA D	LA CB	189	2	100	100	1.4	11.1
53	6	EIA W	EIA D	EIA B	361	0	100	100	2.3	9.4
54	6	EIA D	EIA W	EIA B	361	13	100	100	2.4	9.7

[a] Algorithms 5 and 6 are depicted in Fig. 1.
[b] See Table 1 for abbrevations.
[c] No. of sera with discrepant results in the 1st and 2nd assay to be tested in a 3rd screening assay that differed from the 1st and 2nd.
[d, e] See footnotes *d*, *e*, and *f*, Table 4.
[f] The cost per serum to test *n* sera with the conventional algorithms 1 or 2, divided by the cost per serum to test the same number of sera with the alternative algorithms 5 or 6, respectively.

similar combinations of screening assays in resource-limited settings with different epidemiological patterns of HIV prevalence (*9*, *10*).

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Résumé

Approche simplifiée et moins coûteuse de la confirmation de l'infection à VIH

L'approche classique de recherche des anticorps anti-VIH, qui s'appuie sur la confirmation par Western blot de tous les cas positifs au premier dépistage, est coûteuse. Par exemple, pour examiner les 361 sérums de l'étude, pour lesquels la prévalence des anticorps anti-VIH est de 45%, le coût par test serait de US$ 15,5–25,5.

Lors de cette étude, nous avons utilisé une autre approche dans laquelle les sérums positifs au dépistage initial ont été retestés par une épreuve de dépistage différente, en limitant le Western blot aux sérums ayant donné des résultats contradictoires lors des deux premiers tests.

Cinq titrages immuno-enzymatiques (ELISA) et quatre titrages simples non ELISA à lecture visuelle ont été effectués sur une série de 361 sérums, dont 220 d'origine africaine, 131 d'origine européenne et 10 d'origine sud-américaine; la prévalence du VIH-1 était de 45%. Les résultats obtenus avec différentes paires de titrages ont été analysés rétrospectivement. Le test Western blot HIV-1 de Du Pont a été utilisé comme test de référence.

Pour la plupart des associations, qui comptaient également des associations de titrages non ELISA, la sensibilité et la spécificité étaient de 100%, à un coût en moyenne 6,1 fois plus faible que dans l'approche classique. De plus, on obtenait la même exactitude à un coût 9,0 fois plus

faible que dans l'approche classique lorsque le Western blot était remplacé par un troisième test de dépistage différent des deux premiers.

Dans l'approche décrite ici, il est indispensable que le premier test soit très sensible, puisque cette sensibilité détermine celle de l'association. Pour obtenir une bonne spécificité, il est souhaitable de faire appel à deux titrages basés sur un principe différent et/ou utilisant un type différent d'antigènes. Le fait de retester les sérums positifs par la même méthode ne semblait pas augmenter l'exactitude obtenue pour chaque sérum avec un seul test.

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