An alternative approach to confirming anti-HIV reactivity: a multi-country collaborative study*

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The confirmation of positive screening assay reactions for antibodies to human immunodeficiency virus type 1 (anti-HIV-1) by Western blot is expensive and often gives indeterminate results. We therefore carried out a collaborative study to investigate the confirmation of screening assay reactions using a second screening assay. For this purpose, seven laboratories prospectively tested sequential specimens, using at least one additional screening assay, until about 50 confirmed anti-HIV-1-positive specimens had been identified in each test centre. The reactions of 16 assays were analysed in pairs (assay A and assay B), using assay B on specimens reactive in assay A: A^+/B^+ reactions were considered positive and A^-, negative anti-HIV results. These outcomes were compared with those obtained using confirmatory Western blot. In all, 7950 specimens were tested, and 359 were reported as positive by the laboratories. Within the test centres, eight screening assay pairings gave rise to no false-positive or false-negative results, and these combinations were at least as accurate as a single screening assay followed by Western blot. From 6.3% to 8.3% of the Western blot results were indeterminate.

The number of specimens examined was too small to justify recommending for general use named pairs of screening assays; the choice of these would, in any case, depend on local conditions. However, individual laboratory managers may wish to investigate the large potential savings to be made by confirming HIV infection using a second screening assay on initially reactive specimens. If the more sensitive screening assay is used first, the sensitivity of this approach may be improved by further investigation of specimens that react as A^+B^-.

Introduction

Serological tests for antibodies to human immunodeficiency virus (anti-HIV) are now widely used to screen blood donors, to confirm clinical suspicion of infection, and for surveillance purposes. The sensitivity and specificity of commercially available enzyme-linked immunosorbent assay (ELISA) kits and rapid/simple, instrument-free assays have been well documented (*1*).[a, b] In order to compensate for any lack of specificity in the screening assays, and because of the serious prognostic implications of positive results, a two-tier system of testing has been developed in which sera that are reactive on initial testing are retested with a second (supplemental) test.[c] Although Western blot is the test commonly used for this purpose, it is expensive, is not easy to read or standardize, and often gives indeterminate results. Its cost, use, and interpretation pose difficulties for some laboratories, and there is therefore a need for alternative confirmatory procedures (*2, 3*).[c]

The present study compared the accuracy of using a second screening assay (either ELISA or a simpler test) with that of using Western blot to confirm a positive reaction in an initial screening assay. The study was carried out with the collaboration of seven laboratories in Europe, Australia, and Canada. Altogether, results from ordered pairs of 16 screening assays (68 combinations) were studied to determine whether a second screening assay would be an adequate substitute for a Western blot to confirm positive screening reactions. The outcomes of using either another screening assay or Western blot to confirm reactivity were compared.

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[a] *Operational characteristics of commercially available assays to determine antibodies to HIV-1; report 1.* Unpublished WHO document GPA/BMR/89.4.

[b] *Operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human sera; report 2.* Unpublished WHO document GPA/BMR/90.1.

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[c] *Report of the WHO Meeting on Criteria for the Evaluation and Standardization of Diagnostic Tests for the Detection of HIV Antibody, Stockholm, 7–8 December 1987.* Unpublished document WHO/GPA/BMR/88.1.

Method

In January 1989 seven participating laboratories were invited to test prospectively consecutive serum specimens referred to them until 50 confirmed anti-HIV-positive specimens had been identified in each. Most of the sera had not previously been screened, but in some centres some of the sera had already been tested elsewhere. All the specimens were tested using the assays routinely employed in the centre plus additional assays suggested by the WHO Global Programme on AIDS. Specimens that gave a positive reaction in any assay were tested using Western blot.

The centres were asked to record all the results on every serum and to report the antibody status according to their existing test procedure, i.e., ignoring the results of the additional assays. This procedure generally included the use of one or more screening assays and Western blot; the centre's result (CR) could be positive, negative, or undecided. The analysis of the results was based on the following:

— The assumption that the CR recorded by the participating centre was correct.

— The application of two sets of criteria for scoring the Western blot reactions reported by each centre, either the WHO criteria for positivity (the presence of two of the envelope bands (gp41, gp120 or gp160) or one of these envelope bands plus a band representing antibody to a *gag* or a *pol* gene product)[d] or those proposed by the Centers for Disease Control (CDC) (presence of two of the three bands representing antibody against p24, gp41 or gp120/160). In both schemes any Western blot reaction that did not meet the criteria was scored as indeterminate. If no bands (CDC criteria) or no virus-specific bands (WHO criteria) were reported, the specimen was scored negative.

— The simplification that an "indeterminate" Western blot result or an "undecided" CR was negative, and that equivocal reactions in rapid tests were positive. This was necessary to permit a manageable analysis and presentation of the data. Since the study centres were all in areas with relatively low incidences of HIV infection, the great majority of indeterminate Western blot results and undecided CR specimens were probably anti-HIV negative. Equivocal reactions in rapid tests were regarded as positive in order to test the alternative approach to confirmatory testing as rigorously as possible. The biases that may have been introduced as a result of these simplifications should be borne in mind.

[d] See footnote *c*, p. 751.

The performance of pairs of assays was assessed on the basis of two simple algorithms. The first was as follows: if the initial screening test (A) was unreactive, the final result was negative; if A was reactive, a second screening assay (B) was applied; if B was unreactive the final result was also negative; if A as well as B were reactive, the final result was positive. Thus:

- A^- = negative
- A^+B^- = negative
- A^+B^+ = positive

The second algorithm differed from the first in that A^+B^- specimens were fully investigated, reducing the possibility of a false-negative result. Results obtained upon repeat testing using the same assay were disregarded.

Results

Seven collaborating laboratories provided the results of anti-HIV testing using their current routine, additional assays, and Western blot (Table 1). A total of 7950 specimens were included in the analysis, of which 7516 were primary, while 434 (5.5%) were referred from another laboratory. Three other specimens were excluded: two anti-HIV-2 positive sera, and one sample of cerebrospinal fluid.

For one of the collaborating laboratories, as an example, Table 2 shows the outcome of applying the two algorithms to pairs of anti-HIV assays, and to a single screening assay followed by Western blot (scored by both criteria), as well as the results for single screening assays. The number of tests needed to arrive at an algorithm result varied with the assay pairings and with the order of a given pair. The accuracy of the algorithm results was assessed using the CR as the "gold standard". The proportion of false-negatives is shown as incorrectly negative: total confirmed positive results and the false positives as incorrectly positive: total confirmed negative results.

In some instances, the sensitivity of the second algorithm (further testing of A^+B^- specimens) was greater than that of the first, and the two false-negative proportions are shown separately. The second and fifth columns, taken together, show the number of specimens with outcome A^+B^-, while the second column shows the number of these which by algorithm 1 (i.e., without full investigation) would have given a false-negative result.

There were some discrepancies between the CR and Western blot results (Table 3). Of the 557 sera that were examined by Western blot 503 gave concordant results with the CR using the WHO criteria, while for 495 there was agreement using

Table 1: **Assays used and specimens tested in the study**

Test centre[a]	Assays used	Western blot	No. of specimens: Primary	Referred	Total
CBV	Existing: Elavia HIV 1 Pasteur (Elav1) Wellcozyme HIV 1 recom. (Wellc1) Additional: Ortho HIV 1 Elisa (Ortho) Serodia (SEROD) HIV Chek (HIVCK)	LAV Blot	413	0	413
FCA	Existing: Genetic Systems (GenSys) Additional: Cambridge BioScience (CamBS)	In house	0	49	49
FH	Existing: Abbott HIV 1 recom. (Abb1r) Additional: Wellcozyme HIV 1 recom. (Wellc1)	Biorad	5025	47	5072
ITM	Existing: Abbott recomb HIV 1+2 (Abb1+2) Additional: HIV Chek (HIVCK) Immunocomb (IMMCB)	Du Pont	336	17	353
MVPI	Existing: Enzygnost HIV 1+2 (Enz1+2) Additional: Enzygnost HIV 1 (Enz1) Recombigen (RECOMB)	In house	231	77	308
NBL	Existing: Enzygnost HIV 1+2 (Enz1+2) Wellcozyme HIV 1 recom. (Wellc1) Additional: Abbott HIV 1 recom. (Abb1r) Serodia (SEROD)	Du Pont	1202	130	1332
VRL	Existing: Abbott HIV 1 recom. (Abb1r) Additional: Du Pont HIV 1 recom. (Dup1r) Vironostika HIV Uni-Form (Vuf) Wellcozyme HIV 1 (Wellc1) Serodia (SEROD)	Du Pont	309	114	423
Total			7516	434	7950

[a] CBV = Claude-Bernard Virologie, Paris, France; FCA = Federal Centre for AIDS, Ottawa, Canada ; FH = Fairfield Hospital, Melbourne, Australia; ITM = Institute of Tropical Medicine, Antwerp, Belgium; MVPI = Max von Pettenkofer Institute, Munich, Germany; NBL = National Bacteriological Laboratory, Stockholm, Sweden; VRL = PHLS Virus Reference Laboratory, London, England.

the CDC criteria. No CR-positive sera were negative in the Western blot. On the other hand, four CR-negative sera were positive in the Western blot by WHO criteria and three by CDC criteria. There were 21 undecided CR results reported in the study, compared with 35 indeterminate Western blot results by WHO criteria and 46 by CDC criteria. Equivocal results in the screening assay were rare.

Discussion

In the study the value of using a second screening assay to confirm the presence of anti-HIV was compared with that of using Western blot, taking the test centres own final result (CR) as the "gold standard".

The results obtained show that many pairs of screening assays performed at least as well as a single screening assay followed by Western blot. Moreover, in some cases where ordered pairs of screening assays did give rise to false-positive results, the ELISA absorbance readings were so close to the cut-off value that careful interpretation of the findings of the screening assay would have led to repeat testing or use of a third screening assay. The false-positive results were mostly based on weak reactions; the value of distinguishing between strong

Table 2: **Comparison between the centre's result (CR) and assay pairings, single assays + Western blot (WB) using WHO and CDC criteria, and single assays: an example using the data from the Institute of Tropical Medicine, Antwerp, Belgium**[a]

	Centre's results[b]								
	Positive			Negative			False:		
	A^+B^+	A^+B^-	A^-	A^+B^+	A^+B^-	A^-	Negative (1)[c]	Negative (2)[c]	Positive
Assay pairings									
Abb1+2 → HIVCK	47	2	0	0	11	293	2/49	0/49	0/304
HIVCK → Abb1+2	47	0	2	0	1	303	2/49	2/49	0/304
Abb1+2 → IMMCB	49	0	0	0	11	293	0/49	0/49	0/304
IMMCB → Abb1+2	49	0	0	0	0	304	0/49	0/49	0/304
HIVCK → IMMCB	47	0	2	0	1	303	2/49	2/49	0/304
IMMCB → HIVCK	47	2	0	0	0	304	2/49	0/49	0/304
Single assays + WB									
Abb1+2 → WB (WHO)	49	0	0	0	11	293	0/49	0/49	0/304
HIVCK → WB (WHO)	47	0	2	0	1	303	2/49	2/49	0/304
IMMCB → WB (WHO)	49	0	0	0	0	304	0/49	0/49	0/304
Abb1+2 → WB (CDC)	48	1	0	0	11	293	1/49	0/49	0/304
HIVCK → WB (CDC)	46	1	2	0	1	303	3/49	2/49	0/304
IMMCB → WB (WHO)	48	1	0	0	0	304	1/49	0/49	0/304
Single assays									
Abb1+2						0/49		11/304	
HIVCK						2/49		1/304	
IMMCB						0/49		0/304	

[a] Data from the six other centres are available on request from the author.
[b] One specimen that was WB positive by WHO criteria was indeterminate (and thus classed as negative for this table) by CDC criteria.
[c] By algorithms 1 and 2, respectively.

and weak ELISA reactions and modifying reports accordingly has been emphasized previously (*5*).

In the algorithms used in the study, two interpretations of A^+B^- reactions were explored (either regarding them as negative or investigating them further). The first approach led to a few false-negative results with some pairs of screening assays. Thus, whether in practice it might be justifiable to adopt this approach would depend on the screening assays chosen and on the prevalence of anti-HIV in the population to be tested. However, the algorithm could be supplemented by follow-up testing after an interval of a few weeks to determine whether individuals with A^+B^- reactivity had changed to A^+B^+; if no such change occurred individual would be regarded as anti-HIV negative. The second approach, in which A^+B^- results were investigated further, was sometimes more accurate, but consumed more laboratory resources; nevertheless, its use would still give considerable cost savings because all the A^+B^+ specimens would have been confirmed without having to resort to Western blot.

Several observations should be made about local conditions in the centres which collaborated in this study and about the nature of the assays used. First, when specimens had been selected for referral by another laboratory on the basis of a reaction in a screening assay that was being investigated in a collaborating centre, a false-positive outcome from an algorithm was more likely. Second, it was reported from one centre that a rapid assay had performed better than usual in the course of the study: whatever assays are used for HIV testing, users must be alert to batch-to-batch variations that affect sensitivity and/or specificity. Third, a few pairs of assays, e.g., Abbott and Wellcozyme, appeared to be susceptible to the same nonspecific effects, perhaps because they used a very similar recombinant antigen or had the same format. These considerations, and the fact that no assay pairings were used on enough specimens to establish the accuracy of any of the combinations, meant that there was insufficient information to allow any particular pairs to be recommended. Instead, we suggest that readers select from our

Table 3: **Concordance between centres' final anti-HIV and Western blot results, interpreted according to WHO and CDC criteria, for 557 sera**

Western blot result	Centres' final results		
	No. positive	No. undecided	No. negative
Positive			
WHO[a]	359	3	4
CDC[b]	351	1	3
Indeterminate			
WHO[a]	0	6	29
CDC[b]	8	8	30
Negative			
WHO[a]	0	12	144
CDC[b]	0	12	144

[a] WHO criteria, see footnote *c*, p. 751.
[b] CDC criteria, see ref. *4*.

Table 4: **Screening assay pairings that in at least one study centre did not lead to false results** (the order of their use did not alter the outcome)

Pairing		No. of specimens examined
Abb1r	↔ Enz1+2	1332
Abb1r	↔ SEROD	1332
Abb1r	↔ Wellc1	5072
Abb1+2	↔ IMMCB	353
CamBS	↔ GenSys	49
Elav1	↔ SEROD	1332
Enz1+2	↔ Wellc1	1332
SEROD	↔ Wellc1	1332

results those assay pairings that performed well in this study (Table 4) and use them with specimens of their own whose serostatus has previously been established. If these anti-HIV screening assays give reliable results on a sufficiently large sample of specimens of known status, Western blot confirmation can be omitted, making the testing cheaper and, with machine-read ELISA, more objective.

Further savings might be achieved by considering the order in which the selected pairs of assays are used. With the first algorithm (see Table 2) the outcome of testing using a pair of assays was the same, irrespective of the order in which the assays were used. However, the total number of tests required to achieve the final result depends on the specificity of the first assay, which can vary considerably.

The two simple algorithms used did not incorporate repeat testing (which is often specified in more elaborate algorithms for investigating anti-HIV status) and such testing was not generally carried out. Furthermore, the analysis was concerned only with confirming positivity. In circumstances where the prevalence of HIV is high it may be just as important to confirm negative initial screening reactions by using a second screening assay. For almost all pairs of assays, a double negative outcome would have provided a correct negative result, at least for the specimens examined in the study (results not shown). A further potential advantage of using combinations of screening assays to test and confirm is that fewer specimens will be indeterminate. In the study, 6.3% (by WHO criteria) and 8.3% (by CDC criteria) of specimens examined by Western blot were indeterminate: 83% and 65%, respectively, of such indeterminate specimens were reported by the study centres as anti-HIV negative. It is a disadvantage of the Western blot technique that in many cases it fails to discriminate clearly between seropositivity and seronegativity.

There should be broader investigation of cheap alternative confirmatory strategies for anti-HIV status. For example, very few of the specimens examined in the study were collected in Africa; in other studies, African specimens have often been reported to give false-positive ELISA results. A further difficulty that now confronts confirmatory testing strategies occurs with specimens from populations in which there might be HIV-2 infections. Although combined anti-HIV-1/2 screening assays have become widely available, only two (Abbott and Enzygnost) were included in the present study. The use of a combined screening assay creates the need and HIV-2 but can avoid using two Western blots, which would be inordinately expensive. It may not be easy to devise a simple test algorithm that will identify HIV-1- and HIV-2- positive specimens, distinguishing them from falsely reactive specimens, as well as from each other.

Though it has not been possible to recommend specific alternatives to the use of Western blot for confirming positive anti-HIV reactions, our results show that pairs of screening assays can be just as accurate and give rise to fewer indeterminate (equivocal) results. Laboratory managers who are interested in devoting to other needs the funds which they spend on Western blot strips may therefore want to investigate the savings to be gained by confirming HIV infection through the use of further screening assays on initially reactive specimens.

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Résumé

Une autre façon de confirmer la séropositivité pour le VIH: résultats d'une étude multicentrique

Sept centres collaborateurs de l'OMS ont appliqué de façon prospective leur test habituel de dépistage des anticorps dirigés contre le virus de l'immunodéficience humaine (VIH), et un ou plusieurs tests supplémentaires, à des échantillons de sérum, jusqu'à ce qu'ils aient obtenu environ 50 résultats positifs selon leur protocole de confirmation habituel. Au nombre des tests supplémentaires figuraient diverses épreuves immunoenzymatiques (ELISA) et des tests rapides. Au total, 16 tests de dépistage ont été évalués sur 7950 prélèvements dont 359 se sont révélés positifs après confirmation. Tous les échantillons qui ont donné une réaction positive dans un ou plusieurs tests de dépistage ont été soumis à un Western blot dont la réaction a été interprétée selon les critères proposés par les Centers for Disease Control d'une part et par l'OMS d'autre part.

Dans chaque centre, on a appliqué soit un deuxième test de dépistage, soit la méthode du Western blot pour confirmer la réaction au premier test de dépistage. Les résultats ont ensuite été comparés au résultat final de la procédure habituelle de confirmation, considérée comme étalon de référence. Les résultats obtenus en utilisant successivement deux tests de dépistage (A et B) ont été analysés comme suit: A^- a été considéré comme négatif; A^+B^+ comme positif et A^+B^- soit comme un résultat négatif (algorithme 1), soit comme un résultat nécessitant un nouveau test (algorithme 2). L'application de ces algorithmes a montré que l'utilisation de deux tests de dépistage donnait dans bien des cas des résultats au moins aussi précis que ceux de la confirmation classique d'un test de dépistage unique par Western blot. Huit paires de tests de dépistage n'ont donné aucun faux résultat et les résultats douteux ont été rares. Par contre, la confirmation par Western blot a donné des résultats indéterminés dans 6,3% à 8,3% des cas.

Depuis la fin de cette étude, d'autres tests de dépistage, dont plusieurs permettent de détecter à la fois les anticorps anti-VIH 2 et anti-VIH 1, sont apparus sur le marché. L'application de la technique du Western blot est très coûteuse, surtout lorsqu'elle est utilisée pour confirmer les deux types d'infection, sans être nécessairement plus précise qu'une combinaison de deux ou plusieurs tests de dépistage. Nous pensons que les laboratoires de diagnostic devraient évaluer des algorithmes moins coûteux de confirmation de la séropositivité pour le VIH et les adopter s'ils se révèlent pratiques.

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