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A SIMPLIFIED AND LESS EXPENSIVE STRATEGY FOR CONFIRMING ANTI HIV-1 SCREENING RESULTS IN A DIAGNOSTIC LABORATORY IN LUBUMBASHI, ZAIRE (*)

by

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Summary. — The conventional algorithm for HIV testing based on the confirmation of all positive anti-HIV screening reactions by Western blot (WB) is too expensive for developing countries. We investigated the validity of confirming positive screening assay reactions by a second screening test, limiting the use of the supplemental assay to the discrepant test results (algorithm 3), or screening all sera with 2 different assays and retesting all discrepant results by a supplemental assay (algorithm 4) on a panel of 519 sera in a regional reference laboratory in Lubumbashi, Zaire.

Combining the Vironostika anti-HTLV-III ELISA with HIV Chek 1+2 or Clonatec Rapid HIV 1/2 Ab on all samples and retesting the discrepant results in WB or a line immunoassay (INNO-LIA) (algorithm 4), yielded a sensitivity of 100% and specificities of 98.4% and 99.0% respectively, at costs of 7.3 US$ and 9.3 US$ per test, respectively, for a 40% prevalence of HIV antibody positive samples. The conventional algorithm scored a sensitivity of 97.1% and a specificity of 100% for 11.3 US$ per test. The testing strategy of combining HIV Chek 1+2 and Clonatec Rapid HIV 1/2 Ab, an interesting option for small isolated centra, had a 96.6% sensitivity, but yielded only a slightly better specificity of 99.0%, as compared to 97.8% for HIV Chek alone. The price of combining the two simple assays using algorithm 3 was 6.8 US$ per test, using algorithm 4 was 10.6 US$. HIV testing strategies based on ELISA and a simple HIV test are a valuable alternative for reference laboratories faced with a high prevalence of HIV positive samples.

KEYWORDS: HIV Antibody Assays; ELISA; Simple Rapid Assays; Alternative Confirmatory Strategy.

Introduction

The conventional algorithm for HIV antibody testing consists of a screening assay, followed by supplemental testing of initially reactive samples. Most laboratories use the ELISA as screening assay and confirm the positive samples with Western blot.

However, such an approach is too expensive for the majority of developing countries, including in a situation with a 40% prevalence of HIV-1 positive samples, as encountered in the diagnostic laboratory of the Centre Régional de Lutte contre le SIDA (C.R.L.S.) in Lubumbashi, Zaïre. The method is also time consuming, is insufficiently standardised with regard to procedure and interpretation, and requires highly qualified personnel (6).

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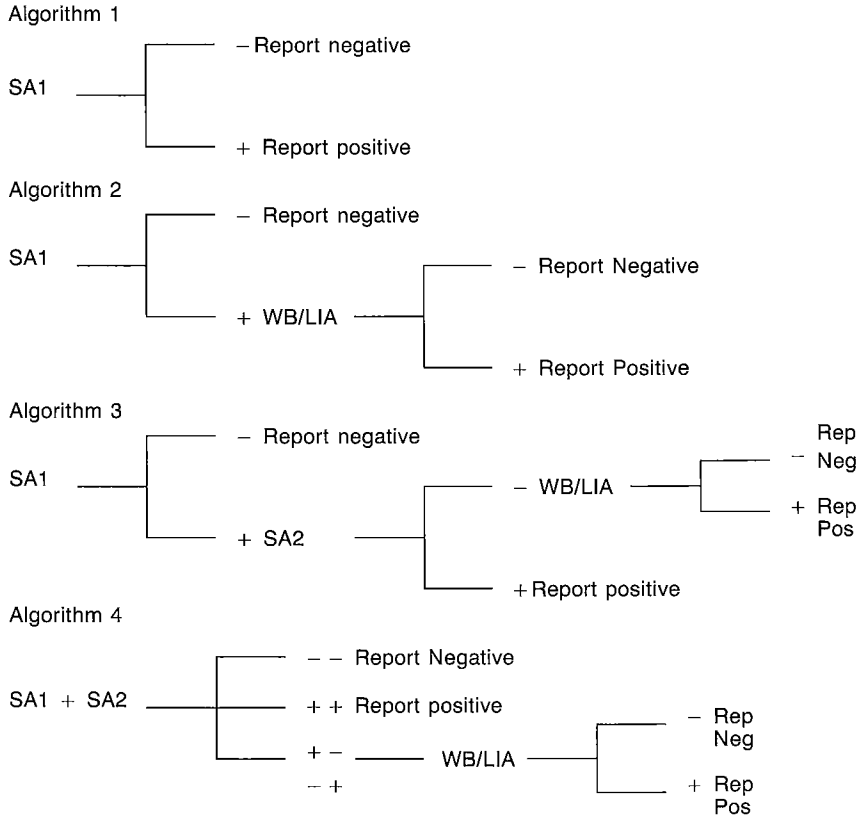
We therefore investigated the alternative of retesting initially reactive samples by a second screening assay, with emphasis on the use of rapid and simple HIV tests with visual reading as second screening assay, and of testing all sera by two different screening assays.The use of a supplemental assay is reduced by confirming only discrepant test results. The exercise was done retrospectively on a panel of 519 sera collected randomly at the regional diagnostic laboratory.

Materials and methods

A panel of 519 primary and referred sera from the diagnostic laboratory of the C.R.L.S., was stored at $-70^{\circ}C$ and thawed once or twice. Sera were mostly from patients presenting with ARC or AIDS like symptoms or from

Figure 1. The four algorithms used.



SA1 : First screening assay
SA2 : Second screening assay

individuals considered to be at high risk for HIV infection. The prevalence of HIV antibody was 39.5%.

All sera were previously tested with ELISA (Vironostika anti-HTLV-III: Organon Teknika) and initially reactives were confirmed by HIV-1 Western blot (Dupont) or a line immunoassay (LIA) (INNO-LIA HIV-1/HIV-2 Ab, Innogenetics). They were retested by two simple, rapid tests with visual reading, including the HIV Chek 1+2 (Dupont) and the Clonatec Rapid HIV-1/2 Ab (Clonatec). The operational characteristics of the different assays used in this study have been extensively described previously (9), with the exception of the Clonatec Rapid HIV-1/2 Ab (Clonatec), which is an immunodot type of assay in which synthetic peptides gp41 and gp36 are used as antigen for HIV-1 and HIV-2 respectively. Sera non-reacting in all three screening assays were considered as true negatives. Samples reactive in one of the screening assays were retested by the LIA, a second generation supplemental assay, showing an excellent overall sensitivity and specificity (100%), when WB results interpreted according to the recent WHO criteria were used as a reference standard (1, 10, 11). Results obtained by the HIV-1 Western blot or LIA were considered as the «gold standard».

The simple assays with visual reading were read independently by 3 persons. Two out of three reading results determined the final result.

The performance and cost of each screening assay and of the combinations of screening assays with supplemental testing were analysed retrospectively. Twelve possible testing strategies were studied, based on four different algorithms (Fig.1).

The sensitivity (true positives/true positives + false negatives) and the specificity (true positives/true positives + false positives) was calculated for each screening assay or test combination. Confidence limits (95% CL) on sensitivity and specificity were calculated according to Bayes' theorem (2). The predictive values of negative and positive test results (NPV and PPV) were calculated for a 39.5% seroprevalence, the prevalence of the panel used, as well as for a 5% seroprevalence, the prevalence found among the general population in Lubumbashi. The cost per specimen for each test or test combination was calculated on the basis of the price charged per test kit (January 1991) (Table 1: A, B, C). For the supplemental assay the price of the LIA (22.6 US$) was taken. Interreader variability for the simple assays with visual reading was expressed as the rate of the number of sera for which test results were differently interpreted by different readers, to the total number of sera tested.

Operational aspects such as initial investment in equipment and training of personnel, transportation and labour are not included in this analysis.

Results

Two sera were labelled as indeterminate in Western blot and excluded from the analysis. From the remaining 517 sera, 204 (39.5%) were confirmed positive for HIV-1 antibodies. None of the sera was positive for HIV-2, though

TABLE 1

Performance and cost of twelve HIV-1 TESTING STRATEGIES (n = 517, 204 positive samples = 39.5 %)

N°	Sensitivity (%) (95 % CL)	Specificity (%) (95 % CL)	FN	FP	NPV (%) 5 %	NPV (%) 40 %	PPV (%) 5 %	PPV (%) 40 %	N° SUPPL. TESTS USED	COST PER SERUM
Algorithm 1: SA1 on all samples										
A VIRONOSTIKA	97,1 (94,8-99,4)	98,1 (96,6-99,6)	6	6	99.8	98.1	72.9	97,1	0	2,4 $
B HIVCHEK	96,1 (93,4-98,7)	97,8 (96,1-99,5)	8	7	99.8	97,5	69.5	96,6	0	4,2 $
C CLONATEC	94,6 (91,5-97,7)	97,4 (95.7-99,2)	11	8	99.7	96,5	65.7	96.0	0	5,8 $
Algorithm 2: SA1 on all samples + C on SA1 reactive sera										
D VIRONOSTIKA + C	97,1 (94,8-99,4)	100 (99,9-100)	6	0	99.8	98.1	100	100	204	11,3 $
E HIVCHEK + C	96,1 (93,4-98,7)	100 (99,9-100)	8	0	99.8	97,5	100	100	203	13,1 $
F CLONATEC + C	94,6 (91,5-97,7)	100 (99,9-100)	11	0	99.7	96,6	100	100	201	14,6 $
Algorithm 3: SA1 on all samples + SA2 on SA1 reactive sera + C on discordant results										
G VIRONOSTIKA + HIVCHEK + C	97,1 (94,8-99,4)	98,4 (97,0-99,8)	6	5	99.8	98,1	76.2	97,5	9	4,5 $
H VIRONOSTIKA + CLONATEC + C	97,1 (94,8-99,4)	99,0 (98,0-100)	6	3	99.8	98.1	83.6	97,5	14	5,3 $
I HIVCHEK CLONATEC + C	96,1 (93,4-98,7)	99,0 (98,0-100)	8	3	99.8	97.5	83.5	98,5	8	6,8 $
Algorithm 4: SA1 on all samples + SA2 on all samples + C on discordant results										
J VIRONOSTIKA + HIVCHEK + C	100 (99,9-100)	98,4 (97,0-99,8)	0	5	100	100	76.7	97,6	17	7,3 $
K VIRONOSTIKA + CLONATEC + C	100 (99,9-100)	99,0 (98,0-100)	0	3	100	100	84.0	98,6	25	9,3 $
L HIVCHEK + CLONATEC + C	96,6 (94,1-99,1)	99,0 (98,0-100)	7	3	99.8	97,8	83.6	98,5	14	10,6 $

SA = screening assay: VIRONOSTIKA ANTI-HTVL-III, HIV CHEK 1+2, CLONATEC RAPID HIV-1/2 Ab.

C = supplemental test: WESTERN BLOT HIV-1 (Dupont) or INNOLIA-1/HIV-2 AB.

FN = FALSE NEGATIVES, FP = FALSE POSITIVES, NPV = NEGATIVE PREDICTIVE VALUE, PPV = POSITIVE PREDICTIVE VALUE, calculated for a 5 % and 40 % seroprevalence respectively.

the Clonatec Rapid HIV-1/2 Ab showed cross reaction on HIV-2 for three of the HIV-1 positive sera. Performance and cost for the twelve testing strategies are depicted in Table 1.

Under these field conditions, the Vironostika anti-HTLV-III, the HIV Chek 1+2 and the Clonatec Rapid HIV-1/2 Ab scored a sensitivity of 97.1, 96.1 and 94.6%, and a specificity of 98.1, 97.8 and 97.4%, respectively. The interreader variability for the HIV Chek 1+2 was 13.1%, and for the Clonatec assay 2.7%.

The sensitivities of the test combinations of algorithm 2 are identical to the ones of algorithm 1 since the combinations start with the same screening assays. However, the conventional testing strategy (algorithm 2) does not produce false positives since we accept by definition that a positive sample in Western blot or LIA is a true positive. The conventional approach is 2.5 to 4.7 times more expensive than the first algorithm, using the same first screening assay.

In algorithm 3, sensitivities range form 96.1 to 97.1% and specificities from 98.4% tot 99.0%. In comparison with algorithm 1, the introduction of a second screening assay for initially positive samples reduced the number of false positives. We are still faced with the same number of false negatives, depending on which assay is used as first assay. This strategy is 1.9 tot 2.5 times cheaper than the conventional algorithm.

Algorithm 4 produced two valuable alternative testing strategies. Retesting all sera by HIV Chek of Clonatec after initial screening by ELISA eliminated all false negatives, while the same specificity as in algorithm 3 is obtained, at a price per serum, 1.5 and 1.2 times cheaper than the conventional algorithm 2.

A combination of two simple tests with visual reading, HIV Chek and clonatec, according to algorithm 3 and 4, yielded a specificity of 99.0%, while the sensitivity did not differ much between the two algorithms. However, a sensitivity of 100%, as obtained when combining the ELISA with the simple assays, was not reached following algorithm 4. Algorithm 3 was 2.1 times cheaper than the conventional algorithm 2; algorithm 4 only 1.4 times.

Discussion

In most developing countries HIV testing imposes a tremendous budgetary burden, especially when the conventional algorithm (algorithm 2, Figure 1) is used. Furthermore, difficulties arise related to standardization and interpretation of Western blot (3, 4, 6).

Our data show that the use of a single HIV antibody screening assay (Vironostika anti-HTLV-III ELISA, HIV Chek 1+2 or Clonatec Rapid HIV-1/2 Ab) in a serum panel in Lubumbashi with a very high (39.5%) HIV antibody prevalence, was capable to correctly identify 97.1%, 96.1% and 94.6% of the 204 positive sera, respectively. Of the 313 negative sera, 98.1%, 97.8%, and 97.4% sera respectively were correctly identified by the above mentioned tests. However, the performance of the screening assays under actual field conditions was somewhat less good as compared to the test performances of the same assays, when performed under ideal laboratory conditions. Similar observations were made in other African countries (5, 7, 8).

In the reference laboratory in Antwerp, the HIV Chek 1+2 and the Clonatec Rapid HIV-1/2 Ab had a sensitivity of 99.3%, and 98.9%, and a specificity of 100%, and 99.0%, respectively.

Taken into account the samble size (n=517) of the Lubumbashi study, the sensitivity and specificity of the 2 simple, rapid tests with visual reading and the more sophisticated ELISA assay, were not significantly different ($p < 0.05$, 95% CL), when tested under field conditions. The latter needs more maintenance and additional infrastructure, and is only suitable when a relatively large number of sera has to be analyzed at a time (> 90 at a time). The simple, non-ELISA tests with visual reading are more suitable for use in small blood transfusion centres and hospitals with a less sophisticated infrastructure, a lower training level of personnel, and where the number of sera to be analyzed are limited. To reduce the number of false negative and false positive results obtained after the use of a single screening assay, additional testing is required. The conventional test alorithm (algorithm 2) confirming initial reactive results with a supplemental assay (WB or LIA) resulted in 100% specificity, but could not exclude false negative results. This conventional algorithm is very expensive though, being 2.5 to 7.1 times more expensive than when a single screening assay was used (algorithm 1).

Two testing strategies, based on the screening of all 517 samples of the panel by both the ELISA and the HIV Chek 1+2 of Clonatec Rapid HIV-1/2 Ab assays, followed by a supplemental assay on all discrepant results (algorithm 4), eliminated all false negatives at a cost respectively 1.5 and 1.2 times cheaper than the conventional approach. The 1.6% and 0.6% of false positives remain a problem, but we are more concerned with the false negatives in our setting, which deals with blood donors.

Algorithm 3, in which all sera, positive in a first screening assay, are repeated in a second screening assay, followed by a supplemental assay on the discrepant results, yielded the same specificity as algorithm 4. The false negatives obtained with algorithm 1 and 2 are not eliminated though. The price was 1.9 to 2.5 times cheaper than the conventional algorithm. Algorithm 4 seems therefore a more interesting strategy than algorithm 3, since it can eliminate a number of false negatives. Furthermore, using this strategy the risk of clerical errors is reduced.

The regional reference laboratory in Lubumbashi serves not only as a diagnostic laboratory, but is also responsible for surveillance activities in the Shaba province, and provides technical support to several AIDS related research projects. The large number of specimens treated in Lubumbashi justifies the use of the rather sophisticated ELISA as screening assay. However, the prevention of blood transfusion related HIV transmission is also one of the major objectives of the C.R.L.S. Urban and rural hospitals throughout the Shaba province are provided with the HIV Chek 1+2 tests, mainly for screening blood donors, but also for initial testing among patients presenting with ARC of AIDS like symptoms.

It was recommended to mail all positive samples to the reference laboratory, but about 50% of the sera never reached Lubumbashi, mainly because of problems related to transportation and communication.

The combination of two simple instrument free and rapid tests (algorithm 3 and 4) is therefore an interesting option for the small and isolated centers

with a low demand for HIV testing. It makes them less dependent from a reference laboratory for supplemental testing and the relative ease with which the tests can be performed make them accessible for health workers with a low educational level. The testing strategies combining HIV Chek and Clonatec were 2.1 (algorithm 3) and 1.4 (algorithm 4) times cheaper than the conventional algorithm, yielding a better specificity than screening with HIV Chek alone. The prices however of these simple tests with visual reading remain unacceptably high. In addition, rigourous internal and external quality are essential components of this approach.

Considering the occurrence of false negative results in the screening assays used, testing all sera by both ELISA and HIV Chek 1+2 or Clonatec Rapid HIV1/2 Ab, and retesting all discrepant results with a supplemental assay (WB or INNO-LIA) is a valuable alternative to the conventional algorithm at the reference laboratory. It is the only algorithm by which the number of false negatives can be reduced. However, its cost is still unrealistically high for the majority of developing countries and its use is not feasable in smaller centers.

More research is needed te develop simple, cheap and reliable HIV antibody tests adapted to the needs of the smaller and isolated centra.

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Une stratégie simplifiée et moins onéreuse pour la confirmation des réactions anti-VIH dans un laboratoire de référence à Lubumbashi, Zaïre.

Résumé. — L'algorithme conventionnel pour le dépistage du VIH, basé sur la confirmation de toutes les réactions anti-VIH positives par un test supplémentaire, le Western blot (WB), est trop cher pour les pays en voie de développement. La validité de deux autres algorithmes a été éprouvée sur un ensemble de 519 sérums dans un laboratoire régional de référence à Lubumbashi au Zaïre. L'algorithme 3 consiste en la confirmation des réactions positives par un premier test de dépistage à l'aide d'un second test, l'usage du test supplémentaire étant limité aux résultats discordants. L'algorithme 4 consiste à tester tous les sérums avec 2 tests différents et à retester tous les résultats discordants à l'aide du test supplémentaire.

La combinaison de l'ELISA Vironostika anti-HTLV-III avec HIV Chek 1+2 ou Clonatec Rapid HIV 1/2 Ab pour tous les échantillons en retestant les résultats discordants en WB ou un «line immunoassay» (INNO-LIA) (algorithme 4), a donné une sensibilité de 100% et une spécificité de 98,4% et 99,0% respectivement, pour une prévalence de 40% d'échantillons, positifs pour la présence d'anticorps anti-VIH. L'algorithme conventionnel a donné une sensibilité de 97,1% et une spécificité de 100% pour 11,3 US $ par test. La stratégie de dépistage en combinant HIV Chek 1+2 et Clonatec Rapid HIV 1/2Ab, représente une option intéressante pour les petits centres isolés. Elle a donné une sensibilité de 96,6%, et une spécificité de 99,0% qui n'était que faiblement supérieure à la spécificité de 97,8% obtenue pour HIV Chek seul. Le prix de la combinaison des deux tests simples utilisant l'algorithme 3 était 6,8 US $ par test, l'algorithme 4 donnait 10,6 US $. Les stratégies de dépistage du VIH basées sur l'ELISA et un test simple constituent une alternative valable pour des laboratoires de référence confrontés à une haute prévalence d'échantillons VIH positifs.

Een vereenvoudigde en minder dure strategie voor de confirmatie van anti-HIV screening reacties in een referentielaboratorium in Lubumbashi, Zaïre.

Samenvatting. — Het conventioneel algorithme voor het testen van HIV, gebaseerd op de confirmatie van alle positieve anti-HIV screening reacties door een supplementaire test, de Western blot (WB), is te duur voor ontwikkelingslanden. We onderzochten de waarde van de confirmatie van positieve screening resultaten door een tweede screening test, waarbij het gebruik van de supplementaire test werd beperkt tot de tegenstrijdige resultaten (algoritme 3), en van het testen van alle sera met 2 verschillende testen, en het confirmeren van alle tegenstrijdige resultaten door een supplementaire test (algoritme 4). Dit gebeurde op een panel van 519 sera in een regionaal referentielaboratorium in Lubumbashi, Zaïre.

Het combineren van de Vironostika anti-HTLV-III ELISA met de HIV Chek 1+2 of de Clonatec Rapid HIV 1/2 Ab op alle stalen en het hertesten van de tegenstrijdige resultaten in WB of een lijn immunotest (INNO-LIA) (algoritme 4), leverde een gevoeligheid van 100 % op, en een specificiteit van 98,4 % en 99,0 % respectivelijk, aan een kostprijs van 7,3 % US $ en 9,3 % US $ per test, respectievelijk, bij een 40 % prevalentie van HIV antilichaam positieve stalen. Het conventioneel algoritme scoorde een gevoeligheid van 97,1 % en een specificiteit van 100 % voor 11,3 % US $ per test. De teststrategie om HIV Chek 1+2 en Clonatec Rapid HIV 1/2 Ab te combineren, een interessante optie voor kleine geïsoleerde centra, gaf een gevoeligheid van 96,6 % maar leverde slechts een iets betere specificiteit op van 99,0 %, vergeleken met 97,8 % voor HIV Chek alleen. De prijs om de 2 eenvoudige testen te combineren volgens algoritme 3 was 6,8 US $ per test, volgens algoritme 4 10,6 US $.

HIV test strategieën gebaseerd op ELISA en een eenvoudige HIV test vormen een waardevol alternatief voor referentielaboratoria die geconfronteerd worden met een hoge prevalentie aan HIV positieve stalen.

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