Journal of Virological Methods, 41 (1993) 213–222

© 1993 Elsevier Science Publishers B.V. / All rights reserved / 0166-0934/93/$06.00

VIRMET 01434

Use of a rapid test and an ELISA for HIV antibody screening of pooled serum samples in Lubumbashi, Zaire

Joseph H. Perriëns[a,b], Kambale Magazani[b], Ngama Kapila[b], Muzita Konde[b], Ungu Selemani[b], Peter Piot[a] and Guido van der Groen[a]

[a]Department of Microbiology, Institute of Tropical Medicine, Antwerp (Belgium) and [b]Centre Régional de Lutte contre le SIDA, Lubumbashi (Zaire)

(Accepted 4 September 1992)

Summary

Pools with a size of 3 and 5 were prepared by mixing one HIV confirmed HIV-1 seropositive serum with either 2 or 4 HIV seronegative sera at the Regional HIV Laboratory in Lubumbashi, Zaire. These pools were assessed in a blind fashion by ELISA (Vironostika anti-HTLV-III microELISA system, Organon Technika). Similarly constituted pools of 3 samples were assayed by a rapid test with visual reading (HIVCHEK 1+2, Dupont de Nemours). With the HIVCHEK, pooling was achieved on the test device itself by dropping consecutively 3 different serum samples on the devices's membrane. After the last serum was soaked in, wash fluid and conjugate were added. Results of the pooling experiments were compared with testing sera individually.

The ELISA results from pools and from individual tested samples matched completely if, and only if, the final dilution of individual samples in the reagent medium was the same as recommended by the manufacturer for testing of individual samples. With the HIVCHEK a sensitivity of 99–100% was obtained with pooled sera. Both approaches seemed sensitive enough to enable their use for screening of blood donors and patient management, but a prospective study to validate these preliminary results is necessary.

HIV antibody assay; ELISA; Rapid test; HIVCHEK; Pooling

Correspondence to: G. van der Groen, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

Introduction

Recently, the steering committee on diagnostics of the Global Programme on AIDS considered pooling of sera as one of the strategies to evaluate for cost effective and appropriate HIV testing (H. Tamashiro, personal communication). Several reports have described testing of serum pools by ELISA (Kline et al., 1989; Emmanuel et al., 1988; Frosner et al., 1990; Behets et al., 1990; Cahoon-Young et al., 1989; El Khoury et al., 1986), and have recommended it for HIV seroprevalence studies. Its use to test blood donors has been advocated, but concerns have been noted about its use in a blood donor screening or diagnostic setting, given that there is a small loss of sensitivity with low titre HIV seropositive samples.

So far, there has been one report describing pooling of up to 5 serum samples and the subsequent testing of serum pools for HIV antibodies using the TEST-PAK (Abbott) and SERODIA (Fudjirebio) rapid tests. This approach was as sensitive and specific as testing serum pools by ELISA (Davey et al., 1991). A second report describes that the HIVCHEK test device (Dupont de Nemours) can be reused once after testing an HIV seronegative sample (Svendsen et al., 1990).

Because the potential for savings on the cost of HIV testing using pooling, we evaluated whether pooling of sera and testing of the pools by ELISA or the HIVCHEK rapid test was sensitive and specific enough to test blood donors for HIV antibodies in the setting of a regional HIV diagnostic laboratory in Lubumbashi, Zaire. Pooling and subsequent testing by ELISA was evaluated using different dilution protocols to establish which dilution protocol yielded optimal results. The use of pooling using the rapid test was assessed because this technique would enable cheaper HIV screening in under-equipped transfusion centers.

Methods

Setting

This study was carried out in the regional HIV diagnostic laboratory of Shaba province, in Lubumbashi, Zaire. This laboratory is operated by the 'Centre Régional de Lutte contre le SIDA' (CRLS) established under an agreement between the government of Zaire and the European Economic Community with technical support from the Institute of Tropical Medicine of Antwerp, Belgium. On average 900 blood donor samples from 6 blood banks in Shaba province are tested each month, with an average HIV seroprevalence of 4.8%.

All tests were carried out using commercially obtained materials. The test kits used were employed routinely in the laboratory.

Use of an ELISA to screen pools of 3 and 5 serum samples for HIV antibodies

Pools were made with well-characterized blood donor sera, which had been stored at −20°C and which had previously been screened for the presence of HIV-1 antibodies by ELISA (Vironostika anti-HLTV III microELISA system, Organon Technika, Boxtel, Holland).

After thawing, samples were tested again by the same ELISA and by line immunoassay (INNO-LIA HIV-1/HIV-2 Ab, Innogenetics, Antwerp, Belgium) (LIA). True HIV seropositive samples were defined as sera being LIA positive (Pollet et al., 1991). True HIV seronegative samples were defined as sera which were ELISA negative and LIA negative, or ELISA positive and LIA negative. These were considered as 'confirmed test samples'. As such, 100 confirmed HIV-1 seropositive and 43 HIV confirmed seronegative test samples were selected. 100 μl of each confirmed 'TEST' sample was pooled with 100 μl of 2 or 4 known HIV seronegative samples (ELISA negative) from blood donors and mixed thoroughly, to yield test tubes containing pools of 3 samples (300 μl) and 5 samples (500 μl) respectively.

A second laboratory technician, who was blinded to the serologic results of the individual sera, tested all pools by ELISA. Pools were tested at two different dilutions. In the first test series, 9 μl of 3 pooled sera and 15 μl of 5 pooled sera, were diluted with 93 and 87 μl sample diluent, respectively, giving a final dilution of 1/34 for each serum in the pool. In the second test series, 3 μl of each pool (regardless of pool size) were mixed with 100 μl sample diluent in the microtiter plate, resulting in a final dilution of 1/103 and 1/172 of individual samples in the test wells for pools of 3 and 5 samples, respectively. ELISA test results were read using an automated photometer and results expressed as positive (optical density (OD) greater than or equal to the cut-off value (CO)), grey-zone ($0.84 < OD/CO < 1.00$), or negative ($OD/CO < 0.85$). All ELISA results on pools were read using the cut-off values for a negative individual serum calculated using the manufacturers' formula for the 1/34 dilution. Pools in which the final dilution of individual sera was 1/103 or 1/172 were also read using the cut- off value as recommended by the manufacturer for testing of an individual serum at 1/100 dilution.

Use of a rapid test (HIVCHEK 1 + 2) to screen pools of 3 serum samples for HIV antibodies

In this evaluation, samples were used that had been screened previously for HIV antibodies by ELISA or HIVCHEK 1 + 2 rapid test (Dupont de Nemours International, Le Grand-Saconnex, Geneva, Switzerland) (HIV-CHEK). They had been stored in cryotubes at −20°C for up to 2 mth. All tests were performed by a laboratory technician who was blinded to the original serology results.

With the HIVCHEK rapid test, pooling was achieved by dropping consecutively 3 different serum samples on the same HIVCHEK device. After

the last serum was soaked in, wash fluid and conjugate were added.

The HIVCHEK test was carried out on series of 3 samples, of which one sample was a confirmed HIV-1 seropositive or a confirmed HIV seronegative 'TEST' sample, and of which 2 were HIV (ELISA) seronegative samples.

Two different test runs were evaluated. In test run A, the confirmed 'TEST' sample was allowed to soak in through the membrane first, followed by the two HIV seronegative samples. In test run B, the reverse order was used.

The HIV-1 seropositive or HIV seronegative 'TEST' samples were derived from 2 groups of patients. The first group of 104 samples was derived from patients suspected of AIDS with a positive ELISA. Of this group of 104 samples, 99 were LIA positive and 5 were LIA negative. They were defined as confirmed HIV seropositive or HIV seronegative test samples according to the LIA results. The second group of 'TEST' samples consisted of 34 ELISA negative samples from blood donors and were defined HIV seronegative by ELISA criteria only.

The HIV seronegative samples, used to mix with the 'TEST' sample, were from consecutive ELISA negative blood donors.

Results

Pooling using ELISA (Table 1)

Overall, 100 HIV seropositive, 43 HIV seronegative, and 1 HIV indeterminate samples were available for testing. The HIV indeterminate sample had an OD/CO of 4.33 in routine ELISA, but by LIA only p17 and p24 bands were present. This 'TEST' sample was detected in all pools, but was excluded from analysis.

There was a complete match between individual testing and pooling when the final dilution of the individual samples in the pool was 1/34 and when the cut-off value was calculated for this dilution. This indicated the sensitivity and specificity of testing pools with this dilution protocol was identical to individual testing.

When pools of 3 or 5 samples were tested at a final dilution of 1/103 and 1/172 respectively, and read using the cut-off value calculated for the 1/100 dilution, one HIV seropositive sample gave a grey-zone result. This sample had OD/CO ratio of 0.99 in pools of 3 and of 0.96 in pools of 5 for an OD/CO ratio of 1.24 when tested individually by ELISA. The use of the cut-off value for the 1/100 dilution on pools of 5, in which the final dilution was 1/172, resulted in the disappearance of 2 grey-zone readings on negative pools. Thus the sensitivity of pooling using this dilution protocol was less, and the specificity higher, than with individual testing.

When the reading on pools of 3 and 5 samples, diluted 1/100 and 1/166, was done using the cut-off value for the 1/34 dilution, 2 and 3 true HIV seropositive samples were missed, respectively. These samples had OD/CO ratio's of 1.24,

TABLE 1

ELISA results on individual samples and on pools

			Test sample HIV seropositive[a] ($n = 100$)	Test sample HIV seronegative[a] ($n = 43$)
ELISA on individual test sample			100 positive	40 negative 2 grey-zone 1 positive
ELISA on pools				
Pool size	Final dilution[b]	Cut-off used[c]		
3 samples[d]	1/34	1/34	100 positive	40 negative 2 grey-zone 1 positive
5 samples[e]	1/34	1/34	100 positive	40 negative 2 grey-zone 1 positive
3 samples	1/103	1/100	99 positive 1 grey-zone	40 negative 2 grey-zone 1 positive
5 samples	1/172	1/100	99 positive 1 grey-zone	42 negative 1 positive
3 samples	1/103	1/34	97 positive 1 grey-zone 2 negative	42 negative 1 positive
5 samples	1/172	1/34	97 positive 3 negative	42 negative 1 positive

[a]HIV serologic status was defined by the line immunoassay result.
[b]Final dilution of each individual sample present in a pool.
[c]Dilution for which the cut-off value was calculated.
[d]1 'test' sample was mixed with 2 HIV seronegative samples.
[e]1 'Test' sample was mixed with 4 HIV seronegative samples.

1.29 and 2.05 when tested individually in routine ELISA, 0.55, 0.59 and 0.99 (grey-zone) in pools of 3 samples, and 0.55, 0.60 and 0.78 in pools of 5 samples. Again, the sensitivity of pooling using this dilution protocol was less, and the specificity higher, than with individual testing.

One HIV negative sample was detected by all pools regardless of the dilution protocol. This sample had an OD/CO of 2.01 when tested individually in ELISA.

TABLE 2

Use of a single rapid test (HIVCHEK 1 + 2) to screen 3 pooled[a] samples for HIV antibodies

	Pool result	Test sample result	
		HIV seropositive +[b] ($n=99$)	HIV seronegative[b] ($n=39$)[c]
Run A[d]	Definitely positive	98 (99%)	0
	Weak positive	0	1 (3%)
	Negative	1 (1%)	38 (97%)
Run B[d]	Definitely positive	99 (100%)	0
	Weak positive	0	1 (3%)
	Negative	0	38 (97%)

[a]Pooling was performed by dropping consecutively 3 different serum samples on the same HIVCHEK device. After the last serum was allowed to soak in, wash fluid and conjugate were added.

[b]HIV serostatus was defined by line immunoassay (LIA).

[c]There were 34 ELISA negative and LIA negative samples and 5 ELISA positive but LIA negative samples.

[d]In run A the test sample was first allowed to soak in through the membrane, followed by the HIV seronegative samples. In run B, the reverse order was used.

Use of a single rapid test (HIVCHEK 1 + 2) to screen pools of 3 samples for HIV antibodies (Table 2)

Of the 99 HIV seropositive 'TEST' samples, 98 (99%) were detected in test run 'A' in which the HIV seropositive sample was introduced first on the test membrane, and 99 (100%) were detected in test run 'B' in which the HIV seropositive sample was introduced last on the test membrane (p = NS). The one pool which gave a discordant result in runs 'A' and 'B' contained one HIV seropositive sample. This sample was positive in HIVCHEK when tested individually and had an OD/CO ratio of 2.01 in ELISA. Test runs 'A' and 'B' have been repeated 3 times on the pool which contained this sample. On the total of 4 test runs 'A' thus performed, 2 were positive and 2 were negative, and all 4 test runs 'B' were positive. The sensitivity of this testing modality was therefore between 99 and 100%.

Of the 39 pools in which only HIV seronegative samples were tested, 38 (97%) gave negative results independent of the order in which samples were introduced on the test membrane. One pool gave a weak positive result. The latter contained 1 sample which gave a weak positive result in HIVCHEK when tested individually, had an OD/CO of 0.39 in ELISA, and was LIA indeterminate (p17 and p24 band weak positive in LIA). The weak positive test signal in HIVCHEK was thus well preserved when this serum was pooled. No technical test failures were observed.

Discussion

Most studies on pooling in which an ELISA was used to screen pools for HIV antibodies used dilution protocols in which the final dilution of individual test samples in the reagent wells was lower than recommended by the test manufacturers, but did not or not completely adjust the cut-off values at which ELISA results were read. These studies found a slightly decreased sensitivity for low titre HIV seropositive samples, or an increased specificity of the pooling procedure. Only one study evaluated pooling of 5 samples with a dilution protocol in which the final dilution of individual samples in the pool was the same as recommended by the test manufacturers for individual testing (El Khoury et al., 1986). In this study, complete agreement between pools of 5 samples and ELISA results obtained on 25 HIV seropositive samples was found using test kits from 4 different manufacturers.

When we evaluated pooling of donor sera to detect HIV seropositive donors using an ELISA, we took into account our routine ELISA protocol in which a 1/34 dilution of individual samples in the test wells is used. When 3 or 5 different serum samples were pooled, so that the final dilution of each sample in the pool was 1/34, as recommended by the manufacturer for testing individual sera, a complete match between pooled and individually tested sera by ELISA was found, as in the study by El Khoury et al. (1986). Pooling of 3 or 5 samples, whereby the final dilution of each test sample was 1/103 and 1/172, and performing the ELISA reading with the cut-off value for the 1/34 dilution resulted in an unacceptable 2 to 3% decrease in sensitivity. Only low-titre HIV seropositive samples escaped detection as could be expected.

With a pool size of 3 and a final dilution of 1/103, ELISA readings with the cut-off value used for the 1/100 dilution resulted in one pool containing a HIV seropositive sample giving a grey-zone reading close to unity. The reason for this was not apparent because final dilution and cut-off corresponded. With a pool size of 5 and a final dilution of 1/172, read at the 1/100 cut-off, the same sample gave a grey-zone reading, but here the explanation might have been that final dilution and cut-off value were not properly matched, which also resulted in the disappearance of 2 grey-zone results on pools which contained HIV seronegative samples only.

When the daily supply of blood donor samples is small and irregular, routine use of pooling and subsequent testing of donor blood by ELISA is not practical. It is not only impossible to operate an ELISA cost effectively at small work loads, but an ELISA is also relatively slow and blood donor serology results need to be available within 24 h. Therefore it was decided to evaluate whether screening of blood donors, using a single HIVCHEK rapid test to screen up to 3 donors at a time, was possible. Pools were constituted directly on the HIVCHEK device because it was considered that this technique would be most useful in under-equipped transfusion centers where even the racks, tubes and pipettes, needed to constitute pools otherwise may be lacking and where the technical skills for meticulous record keeping are often not available. In

addition, this prevents increased reagent consumption associated with attempts to reuse used HIVCHEK devices to test new samples (Svendsen et al., 1990).

This study documented that the use of a single HIVCHEK test to test pools of 3 samples is as sensitive (between 99 and 100%) as screening of individual samples. The reason why one of the 99 HIV seropositive 'TEST' samples was not detected in one of the test runs was unclear.

The specificity of testing serum pools by ELISA or HIVCHEK could not be assessed reliably in the present study because of limited sample size. In the ELISA test, matching the final dilution of individual pooled samples to the one recommended for individual testing results in a reagent medium containing more serum than recommended. In the present study the total serum dilution in the reagent wells containing pooled serum was 9/103 (1/11) for pools of 3 samples, and 15/102 (1/7) for pools of 5 samples, when the final dilution of individual samples was 1/34. This should lead to a decreased specificity on pools, because the reagent medium contains more proteins than recommended. In another study on over 2000 pools containing 5 sera each, a decreased specificity of 94.6% (Organon Vironostika) to 99.3% (ELAVIA 1, Pasteur) was described, compared to 99.38% to 99.57% with individual testing using corresponding test kits (El Khoury et al., 1986).

Pooling has been advocated for studying HIV seroprevalence because of the potential of important savings on reagents and labor. A computer model to calculate optimum pool size for different levels of labor cost and HIV seroprevalence has been presented which showed that pooling remained very cost effective in developing countries as long as HIV seroprevalence remained under 10% (Behets et al., 1990). This model does not take into account how decreased specificity might influence the cost of the pooling procedure. However, as a false positive pool has the same effect on labor and reagent consumption as a real positive pool, HIV seroprevalence can be substituted in the model with the sum of HIV seroprevalence and false positive test rate on pools. At the lowest specificity of 94.6% recorded by Khoury et al. (1986) pooling is thus likely to remain cost-effective in developing countries as long as HIV seroprevalence does not exceed 5%, and higher if specificity is better. This is the case in most of Zaire where HIV seroprevalence in the general adult population does not yet exceed 5% (Nkowane, 1991), and could even be achieved in Kampala, where up to 30% of urban adult population is HIV seropositive (Guay et al. (1990), through selective blood donor recruitment (P. Kataaha, personal communication). However, more data on the specificity of pooling are required to give a reliable assessment of the cost-effectiveness of pooling in the blood donor setting.

In summary, it was documented that pooling of 3 or 5 serum samples and subsequent testing of the pools by the Organon Technika Vironostika HTLV III microELISA system was as sensitive as testing of individual samples by the same ELISA if, and only if, the final dilution of individual pooled samples in the reagent medium was the same as the one recommended by the test manufacturer for individual testing. If this precaution was disregarded, a small

loss of sensitivity resulted. While the specificity of pooling using this dilution protocol is likely to be slightly less than individual testing, it is in our opinion the only suitable pooling protocol using this ELISA test in a blood donor setting. In addition, it was shown that one HIVCHEK rapid test device can be safely used to test pools of 3 serum samples for HIV antibodies whereby the pooling was achieved by dropping consecutively 3 different serum samples on the test device, followed by washing and adding the conjugate. This pooling system is very simple and economical and avoids the use of dilution tubes and special pipette devices. Both procedures seemed sensitive enough to test blood donors for HIV antibodies. However, as the above results on the sensitivity of pooling were obtained with artificially made pools, their prospective confirmation is needed. To enable a better assessment of the cost-effectiveness of pooling, its specificity under field conditions should also be determined.

Acknowledgements

The authors wish to thank the European Economic Community, Directorate General VIII, for financial support.

References

Behets, F., Bertozzi, S., Kasalki, M., Kashamuka, M., Atikala, L., Brown, C., Ryder, R.W. and Quinn, T.C. (1990) Successful use of pooled sera to determine HIV-1 seroprevalence in Zaire with development of cost-efficiency models. AIDS 4, 437–741.

Cahoon-Young, B., Chandler, A., Livermore, T., Gaudino, J. and Benjamin, R. (1989) Sensitivity and specificity of pooled versus individual sera in a human immunodeficiency virus antibody prevalence study. J. Clin. Microbiol. 27, 1893–1895.

Davey, R.J., Jett, B.W. and Alter, H.J. (1991) Pooling of blood donor sera prior to testing with rapid/simple HIV test kits. Transfusion 31 (suppl), 7S.

El Khoury, A.B., Muller, A. and Richard, D. (1986) Anticorps anti-LAV/HTLV III chez les donneurs de sang: résultats préliminaires du dépistage en pool de 5 échantillons. Rev. Fr. Transfus. Immuno-hématol. 24, 183–192.

Emmanuel, J.C., Bassett, M.T., Smith, H.J. and Jacobs, J.A. (1988) Pooling of sera for human immunodeficiency virus (HIV) testing: an economical method for use in developing countries. J. Clin. Pathol. 41, 582–585.

Frösner, G.G., Dobler, G. and Falkner von Sonnenburg, F.J. (1990) Cost reduction of unlinked testing for anti-HIV by investigation of pooled sera. AIDS 4, 73–75.

Guay, L., Mmiro, F., Ndugwa, C., et al. Perinatal outcome in HIV-infected women in Uganda. VIth International Conference on AIDS. San Francisco 1990. Abstract Th.C.42.

Kline, R.L., Brothers, T.A., Brookmeyer, R., Zeger, S. and Quinn T.C. (1989) Evaluation of human immunodeficiency virus seroprevalence in population surveys using pooled sera. J. Clin. Microbiol. 27, 1449–1452.

Nkowane, B.M. (1990) Prevalence and incidence of HIV infection in Africa: a review of data published in 1990. AIDS 1991; 5 (suppl. 1): S7–S15.

Pollet, D.E., Saman, E.L., Peeters, D.C., et al. (1991) Confirmation and differentiation of antibodies to human immunodeficiency virus 1 and 2 with a strip-based assay including recombinant antigens and synthetic peptides. Clin. Chem. 37, 1700–1707.

Svendsen, J., Faber, V. and Maselle, S.Y. (1990) Re-use of 'HIV-check' in developing countries. Lancet 336, 1198.