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Evaluation of Human Immunodeficiency Virus Seroprevalence in Population Surveys Using Pooled Sera

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The pooling of individual serum samples to determine human immunodeficiency virus (HIV) seropositivity was examined to assess whether testing pooled sera was technically feasible, cost-effective, and accurate for estimating seroprevalence in large population surveys. The sensitivities and specificities of three commercially available HIV enzyme-linked immunosorbent assay (ELISA) kits were tested using 65 serum pools of 15 individual serum samples each (975 total serum samples) at two different dilutions. With pooled sera, the Organon Teknika Bio-EnzaBead ELISA at half the dilution recommended by the manufacturer showed the best agreement with ELISA and Western blot results of individual sera. In subsequently testing 92 pools, each containing 15 individual serum samples from a population of American patients attending a sexually transmitted diseases clinic, the estimated seroprevalence was 5.27 compared with 4.93% in a test of 1,380 individual serum samples and 5.19% in a test of 4,028 individual serum samples from the same population. In an evaluation of 1,380 African patients using 10 serum samples per pool, the estimated seroprevalence was 5.79 compared with 6.16% in a test of individual sera. These results indicate that ELISA testing with pooled sera is highly sensitive and specific and appears to be a cost-effective means for estimating HIV seroprevalence in large population-based surveys.

The standard procedure for screening individuals for antibody to the human immunodeficiency virus (HIV) is currently by enzyme-linked immunosorbent assay (ELISA) followed by Western (immuno-) blot confirmation (3, 4, 7). Although ELISA screening of blood is one of the least expensive technologies for detection of HIV infection, it can be relatively expensive for many developing countries where the amount of funds per capita for medical purposes are less than the cost of a single ELISA for HIV antibodies (8). One possible method of lowering the cost of HIV testing, particularly in large population surveys, involves pooling of the sera from several individuals and testing the pool for HIV infection by ELISA. The testing of pooled sera has been used successfully in the past for identifying individuals with syphilis (5). The reduction in the number of tests afforded by pooling may drastically reduce the overall laboratory costs of a serologic survey. However, it is also dependent upon the ability of the antibody assay to maintain specificity and sensitivity with pooled samples. This study was undertaken to assess whether the testing of pooled sera is technically feasible, cost-effective, and an accurate method for determining seroprevalence in large-population-based surveys.

MATERIALS AND METHODS

A series of experiments were performed to estimate the reliability of HIV ELISAs using pooled sera. Sera collected at an inner-city sexually transmitted disease clinic in Baltimore, Md., were pooled in groups of 15. Sixty-five pools were each prepared by pipetting 10 μl of each individual serum from a total of 975 individual serum samples. The sera pools were then tested by three commercially available ELISAs for detection of HIV antibody at different dilutions. The assays used included the Bio-Enzabead ELISA (Organon Teknika, Charleston, S.C.), the HTLV III ELISA (Du Pont Co., Wilmington, Del.), and the HTLV III ELISA (Abbott Laboratories, Abbott Park, Ill.). The dilutions used for pooled sera were the standards specified by the manufacturers for individual serum samples and half the specified dilution. The final dilution of each individual serum sample ranged from 1:157.5 to 1:6,615, depending on which assay was used. The individual serum samples were randomly selected for combining into pools. The number of positive sera within each pool varied from zero to three. Assays were then performed according to specifications of the manufacturers. Individual serum samples were also tested by ELISA (Organon Teknika) and all reactive sera were confirmed by Western blot analysis (Du Pont). A Western blot was considered positive if *gag* (p17 and p24), *pol* (p31 and p64), and *env* (gp41, gp120, and gp160) antigens were present.

In addition, sera from two large-population surveys (one American and the other African) were analyzed by using the pooling method. HIV seroprevalences of the two populations were estimated and compared with the seroprevalences derived by testing individual serum samples. The American population consisted of patients attending a sexually transmitted diseases (STD) clinic in Baltimore, Md., and the African population consisted of health care workers employed at Mama Yemo Hospital in Kinshasa, Zaire. A total of 1,380 individual serum samples from the STD clinic population were pooled into 92 pools of 15 samples each. Because of a higher expected seroprevalence in the African population, the African sera were pooled into 138 pools consisting of 10 serum samples each as well as 92 pools of 15 serum samples each. The pools were analyzed by using the Organon Teknika Bio-EnzaBead ELISA at a dilution of 1:46. Otherwise, the assays were performed according to the specifications of the manufacturers. Sera were also tested individually as described above.

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TABLE 1. Comparison of different commercially available ELISA kits used for detection of antibodies to HIV in pooled sera

Manufacturer	Dilution	Pool result	No. of positive sera in each pool			
			0	1	2	3
Organon Teknika	1:17	Positive	1	24	4	1
		Negative	34	1	0	0
	1:46	Positive	0	25	4	1
		Negative	35	0	0	0
Du Pont	1:21	Positive	0	23	4	1
		Negative	35	2	0	0
	1:10.5	Positive	0	24	4	1
		Negative	35	1	0	0
Abbott	1:441	Positive	0	23	4	1
		Negative	35	2	0	0
	1:220.5	Positive	6	24	4	1
		Negative	29	1	0	0

The seroprevalence, $\hat{p}$, from analysis of pooled sera was estimated by the following method. If the number of pools is n, each pool is composed of an equal number of serum samples c, and the total number of positive pools is denoted by s, then the maximum likelihood estimate of $\hat{p}$ is given by the formula $\hat{p} = 1 - (1 - [s/n])^{1/c}$. The asymptotic variance of $\hat{p}$ is estimated by $v = \{s/n[1-(s/n)]^{[2/c]-1}\}/c^2n$. An approximate 95% confidence interval for $\hat{p}$ is therefore calculated by $\hat{p} \pm 2\sqrt{v}$.

RESULTS

In the first study, testing of 975 individual serum samples from the Baltimore STD clinic population resulted in 36 seropositive samples. After randomly combining the 975 sera into 65 pools, 30 of the 65 pools contained at least one positive individual serum sample (25 pools contained exactly one positive serum sample, 4 pools contained exactly two positive serum samples, and 1 pool contained exactly three positive serum samples). The results based on testing the pooled sera are shown in Table 1.

All three assays at all dilutions correctly detected as positive all pools which contained two or more positive serum samples. When the pools contained only one positive serum sample, only the Organon Teknika assay at a dilution of 1:46 agreed 100% with results obtained by testing each individual serum sample separately by ELISA and Western blot. The only false-positive pools were detected by the Organon Teknika assay at a 1:76 dilution and by the Abbott assay at a 1:220.5 dilution. False-negative results were probably caused by increasing the dilution of each positive serum sample 7.5- or 15-fold. Although the kits are extremely sensitive for their initial purpose (screening of blood supply), two borderline-positive samples diluted 7.5- and 15-fold were not detected by all assays. It was felt that false-positive results obtained in the Abbott assay were caused by nonspecific binding due to doubling the amount of serum normally used when performing the assay.

In the analysis of pooled sera from two additional population groups, 92 pools were first made from 1,380 American patients attending an STD Clinic in Baltimore, Md. (Table 2). In testing the sera individually, it was determined that 51 of the pools contained positive serum samples. The number of positive serum samples in each of the 51 pools varied from one to three. The ELISA identified all 51 pools, and there were no false-positive pools detected (Table 2). The estimated seroprevalence of the American STD population was calculated to be 5.27% with a 95% confidence interval of 3.8 to 6.7%, compared with the actual seroprevalence of 4.93% in a test of 1,380 individual serum samples and 5.19% in a test of 4,028 individual serum samples from the same population (Table 2).

Similarly, 92 pools of 15 serum samples from 1,380 African health care workers were assayed. It was first determined from analysis of individual serum samples that 50 of the pools contained positive sera (Table 2). The number of positive serum samples in each of the 50 pools varied from one to four. The ELISA identified all 50 positive pools, and there were no false-positive pools detected. The estimated seroprevalence of the African population was calculated to be 5.11%, compared with an actual seroprevalence of 6.16% obtained by using individual serum samples (Table 2). Due to the expected high seroprevalence of HIV in 1,380 African patients, the African sera were also pooled in groups of 10 serum samples per pool. Of the 138 pools, 61 pools contained positive serum samples that varied from one to three per pool as determined by individual ELISA and Western blot analysis. The ELISA detected all 61 positive pools and 1 false-positive pool (Table 2). The estimated seroprevalence obtained by using 10 serum samples per pool of the African sera was 5.79%, compared with the actual seroprevalence of 6.16% obtained in a test of 1,380 individual serum samples (Table 2).

DISCUSSION

The Organon Teknika ELISA at a dilution of 1:46 correctly identified as positive all serum pools with at least one positive serum sample and correctly identified as negative all pools with no positive serum samples. These results demonstrated that using pooled sera with a commercially licensed ELISA was technically feasible. The only change from the instructions of the manufacturers was the use of half the specified dilution when testing pooled sera from either 10 or 15 individual samples. It was important that few changes

TABLE 2. Calculation of the estimated seroprevalence of HIV infection obtained by using pooled sera in two populations

Population and no. of serum samples per pool	No. of:					Seroprevalence (%)		95% confidence interval (%)[a]
	Pools	Serum samples (total)	Positive pools detected	False-positive pools	False-negative pools	Individual serum samples	Pooled sera	
American, 15	92	1,380	51	0	0	4.93	5.27	3.8–6.7
African								
15	92	1,380	50	0	0	6.16	5.11	3.7–6.6
10	138	1,380	62	1	0	6.16	5.79	4.3–7.2

[a] Based upon pooled sera.

were made from the specifications of the manufacturers, since training of personnel would be consistent and the chance of technical error would be markedly reduced. Since all of the reactive individual serum samples in the study were Western blot reactive and since there were few borderline ELISA-reactive, Western blot-positive samples, the ability of the pooled sera assay to consistently detect low-level antibody samples was not rigorously tested. While this needs further evaluation in order to determine whether pooling of sera can successfully be used to identify all infected individuals, it is well known that the majority of Western blot-reactive samples are usually strongly reactive in all commercially available ELISAs.

Different levels of agreement with ELISA and Western blot were observed when three commercially available HIV ELISAs using pooled sera were compared. The Organon Teknika Bio-EnzaBead ELISA had the highest percentage agreement, followed by the Du Pont HTLV III ELISA. With the dilutions used in this study, the results obtained by the Abbott HTLV III ELISA using pooled sera were not as optimal as those observed with the Organon Teknika assay. It is possible that different pooled dilutions or other small procedural changes would improve the sensitivity and specificity of both the Du Pont and the Abbott assays. Further validation of sera pooling by using these and other assays should be done before large serum surveys are conducted with unevaluated ELISAs of pooled sera.

The geographic origin of the sera did not affect the results obtained by pooling sera. Sera from an African population was specifically chosen because of previous reports of nonspecific reactivity of African sera (1, 2) and because of the potential utility of using pooled sera in developing countries. Serologic results were comparable whether the pooled sera came from an American or an African population.

The major benefit of screening pooled sera rather than individual serum samples for antibody to HIV is to determine seroprevalence of HIV infection in large-scale population-based serum surveys at a markedly reduced cost. Determining the seroprevalence of HIV infection among STD clinic patients by using pooled sera gave results comparable with the actual seroprevalence determined by analysis of individual serum samples. It is of interest that the estimated seroprevalence of HIV infection in patients attending a STD clinic was 5.27% with 92 pools of 15 individual serum samples each (1,380 sera), which was nearly identical to the 5.19% calculated from analysis of 4,028 individual serum samples. Thus, the estimated seroprevalence from pooled sera was in this case more predictive of the overall population seroprevalence than the calculated seroprevalence obtained by testing 1,380 individual serum samples. This determination of seroprevalence by using pooled sera cost 15-fold less than ELISAs for the individual sera. The cost efficiency of using pooled sera increases dramatically as the size of the pool and the total number of sera needed to be analyzed increase. However, as the seroprevalence increases in a particular population, the accuracy of pooling and hence its cost-effectiveness decrease, since smaller pools consisting of fewer individual samples are required to maintain accurate seroprevalence estimates.

The efficiency of analyzing a given number (n) of serum pools instead of an equal number (n) of individual serum samples is displayed in Table 3 for a variety of true seroprevalences. Here, efficiency is defined to be the ratio of the variance of the prevalence estimate from n individual sera to the variance of the prevalence estimate from the n pooled sera. For example, when the true prevalence is 0.05, the calculated prevalence from testing n individual sera is 10 times less efficient than calculating the prevalence estimate by using n pools containing 15 sera each.

TABLE 3. Theoretical efficiency of prevalence estimate obtained by testing pools of sera for different HIV seroprevalences and pool sizes

HIV seroprevalence	Efficiency of prevalence estimate[a] with a pool size of:		
	5	10	15
0.0001	4.99	9.99	14.98
0.001	4.99	9.95	14.89
0.01	4.90	9.55	13.96
0.02	4.80	9.11	12.97
0.05	4.50	7.85	10.22
0.10	4.00	5.94	6.48
0.25	2.59	1.98	1.01
0.50	0.80	0.09	0.00

[a] Efficiency is defined as the ratio of the asymptotic variance of the prevalence estimate obtained from n individual serum samples to the variance of the prevalence estimate obtained from n pooled sera.

The African population had a higher seroprevalence than the American population, and thus a smaller pool size gave a more precise estimate of the actual seroprevalence. The 95% confidence intervals were wide in these studies due to the small size of the populations studied. Results of this study indicate that if the seroprevalence is expected to be 5% or less, then pools of 15 can be used. This adjustment in pool size will provide the most accurate results and maintain cost efficiency.

There is the potential to use pooled sera for diagnosis of HIV infection in individual patients or in screening of potential blood donors in developing countries. Emmanuel et al. recommend using pooled sera to screen blood donors in countries where the HIV seroprevalence is less than 10% (6). In these circumstances, an ELISA-positive pool would need to have its individual serum components reanalyzed separately. However, seronegative pools would not need to be retested, resulting in improved cost efficiency. However, a borderline-reactive sample may not be detected in a pooled sample. In conducting population-based serologic surveys, reanalysis of individual serum samples may not be required, particularly if they were collected anonymously, thus resulting in an enormous savings in cost, labor, and time when pooled sera are used.

In the United States and other developed nations, using pooled sera for screening blood donors is not necessary for economic reasons and would not be as safe as methods used now. However, these countries could use pooled-sera assays to determine HIV seropositivity in surveillance studies of different populations. By using pooled sera, not only would the costs of such studies be tremendously reduced, but confidentiality would be assured.

In a preliminary field study in Africa, the estimated seroprevalence obtained by using 250 pools of 10 serum samples each was 3.6%, which is currently identical to the 3.6% seroprevalence as determined by analysis of 2,500 individual serum samples (F. Behets et al., personal communication). This study will be continued until 10,000 individuals have been completely analyzed by both methods in order to validate the efficacy of pooling sera for the detection of HIV antibody in the field. It is therefore hoped that the reliability of serum pooling for HIV screening will provide

public health officials with a cost-effective means of determining the extent of HIV infection in selected populations.

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