

Sensitivity and Specificity of Pooled versus Individual Sera in a Human Immunodeficiency Virus Antibody Prevalence Study

BARBARA CAHOON-YOUNG,¹ ANN CHANDLER,^{1*} TIMOTHY LIVERMORE,² JAMES GAUDINO,² AND ROBERT BENJAMIN²

Public Health Laboratory¹ and Communicable Disease Division,² Alameda County Health Care Services Agency, Oakland, California 94607

Received 14 November 1988/Accepted 9 May 1989

We evaluated the efficacy of testing pooled versus individual sera for the detection of human immunodeficiency virus antibody. A total of 5,000 individual specimens and 500 pools of 10 specimens each were assayed by an enzyme-linked immunosorbent assay. There was complete agreement in human immunodeficiency virus enzyme-linked immunosorbent assay reactivity for pooled versus individual specimens. An estimated savings of 60 to 80% (labor and supplies) can be realized dependent upon pooling and assay format.

Given the national need to perform widespread population-based seroprevalence studies and the concomitant high cost of testing sera individually, it is essential that a cost-effective method of testing large numbers of specimens be developed. Ideally, the method would not compromise specificity or sensitivity. Toward this end, we set out to determine a more economical basis for screening large numbers of serum samples, particularly those from low- to moderate-risk populations. Screening pooled sera followed by testing of individual sera from antibody-positive pools could substantially reduce the effort and costs required to screen many low- to moderate-risk individuals. To our knowledge, there are no published accounts that attest to the suitability of pooled sera in the routine detection of human immunodeficiency virus (HIV) antibody prevalence. Investigators have shown, however, that serum diluted 30-fold continued to demonstrate reactivity when tested for HIV antibodies in an enzyme-linked immunosorbent assay (ELISA) (10). Additional studies have found HIV antibodies in samples of pooled control sera (2, 12) and pooled immunoglobulin tested for safety concerns (5, 8). Numerous other studies attest to the extreme sensitivity and specificity of the commercially available HIV ELISAs (1, 7, 10, 11, 14), which approach 100% when these results are combined with results from supplemental assays such as the immunofluorescence assay (IFA) and/or Western blotting (WB) (1). In the present study, we evaluated the reliability and cost efficiency of testing pooled versus individual sera for the detection of HIV antibodies.

Unlinked HIV antibody assays were performed with sera routinely obtained from sexually transmitted disease clinics in Alameda County. The pool size (10-fold) was determined after making serial twofold (1:2 to 1:256) dilutions of the HIV ELISA-reactive specimens (HIV ELISA kit from E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Pools were generated within 3 days of assay by inoculation of 50 μ l of 10 randomly sampled individual specimens into a storage vial followed by thorough mixing with pooled nonreactive sera as a diluent. A total of 5,000 individual and 500 pooled specimens were then assayed.

The HIV ELISA was performed and interpreted by using the automated procedure and equipment supplied by the manufacturer (du Pont). Both the reactive threshold value

(cutoff) and the recommended grey zone ($0.85 \times$ the cutoff) were used in the pooling protocol to evaluate the sensitivity and specificity of pooling without modifying current procedures. For assay, 80 individual specimens, eight corresponding pools, and eight controls (four positive, two negative, and two blanks) were used in each microdilution plate.

Slides for the IFA of HIV antibodies were supplied by the Viral and Rickettsial Disease Laboratory, State of California Department of Health Services. Slide preparation and the assay have been described elsewhere (7). In addition, WB was performed and interpreted according to manufacturer's instructions (du Pont).

Individual specimens showing repeat reactivity in the ELISA were further tested by the IFA. Only if they were reactive in the ELISA and IFA were the specimens considered positive (7, 11, 13). However, individual sera with discrepant ELISA and IFA results (reactive ELISA, nonreactive IFA) were designated as probable false-positives (FPs) and were further tested by WB to resolve the discrepancy. All probable FPs found nonreactive or indeterminate by WB were then considered FPs (1, 6, 9, 11). No further follow-up for specimens with conflicting results was possible because of the unlinked protocol.

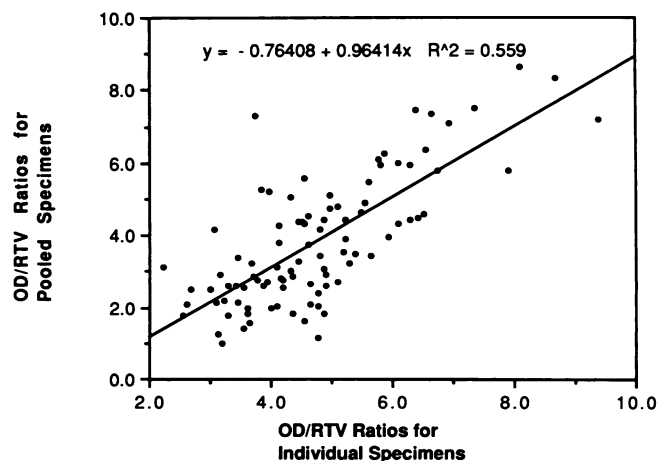


FIG. 1. Plot of reactivity ratios (OD/RTV ratios) for individual versus pooled specimens.

* Corresponding author.

TABLE 1. Estimated savings resulting from pooling^a

Testing protocol	No. of initial specimens tested	No. of repeat (reactive) specimens tested (duplicates)	Total no. of specimens tested	No. of reaction trays ^b (rounded-off value)	Estimated cost (\$) ^c	Estimated savings (%)
Individual sera	5,000	208	5,208	59.18 (60)	8,928.00	
Pooled sera (followed by individual reactive sera)	500 (950)	208	1,658	18.84 (19)	2,827.20	68
Pooled sera not followed by individual reactive sera (hypothetical)	500	190	690	7.84 (8)	1,190.40	86

^a Estimates include costs of assay materials and labor. Each pool included 10 serum specimens.

^b Each tray held 88 specimens.

^c The cost for each tray was \$148.80.

Since, in the present study, HIV ELISA of pooled sera was used as a screening procedure for HIV antibody positivity in a large, relatively low-prevalence population, pool reactivity was designated on the basis of ELISA results without further testing by the IFA or WB.

Estimates of the costs of supplies and laboratory staff time were based on our laboratory's current costs assuming that several hundred specimens would be available for pooling and testing. Calculated cost estimates for two different pooling protocols were derived, one by assuming that each constituent specimen in reactive pools would then be individually tested and another by assuming a hypothetical protocol that would omit individual testing of constituent specimens in reactive pools.

In this study, the overall HIV antibody positivity rates were 2.08% (104 of 5,000) for individual specimens and 19% (95 of 500) for pooled specimens. This approximate 10-fold difference is consistent with pool size. Slight rate differences resulted from pools containing two (7 of 500) or three (1 of 500) reactive specimens. None of the pools contained more than three reactive specimens.

We found complete agreement in antibody reactivity in pooled versus individual sera. In addition, since pools were found to be reactive only when the individual specimens were true-positives, the pooled-serum HIV antibody screening protocol followed in this study seems justified. The detection of a few false-positives (7 of 5,000) among individual specimens was due in part to the extreme sensitivity of the HIV ELISA, as well as the stipulated cutoff value. These sensitive assay conditions have the advantage of detecting very low levels of HIV-specific antibodies. However, even low levels of cross-reacting antibodies have been shown to result in a lowered specificity (3, 4, 11).

A comparison of the reactivity ratios (specimen optical density/cutoff optical density) for reactive pooled sera with those for the corresponding individual sera demonstrated a good correlation between pooled and individual ratios, suggesting a linear relationship (Fig. 1). For pools with more than one reactive serum specimen, the average reactivity ratio was plotted. The reactivity ratio was inexplicably higher for a few pooled sera than for any individual sera within those pools. In a few other pools, an unaccountable decrease in reactivity after pooling was observed. Nevertheless, 100% agreement between pooled and individual constituent final HIV antibody test interpretations remained. Overall, the reactivity ratios for pooled sera relative to those for the corresponding individual sera ranged from a low of 0.3 to a high of 1.36.

In this study and past reports (1, 6, 9, 11), specimens with low levels of repeat reactivity in the ELISA but nonreactive in the IFA and negative or indeterminate by WB have been

designated FPs. While we found several FPs among non-pooled specimens (7 of 5,000; 0.14%) having characteristically low reactivity ratios (less than 2.0), the pools containing these FP specimens were all nonreactive in the ELISA, suggesting a specificity improvement when using the HIV ELISA to screen a population with a pooled-serum protocol, but further studies are recommended. In no other cases did we find discrepancies, either FP or false-negative, in pooled versus individual sera.

Finally, we found an approximately 60 to 80% savings in labor and materials when a pooled-serum protocol was used for HIV seroprevalence monitoring (Table 1). To calculate savings estimates for each pooled-serum protocol as compared with an individual-serum protocol for our laboratory, we established a unit cost for both labor and materials for each HIV ELISA tray based on our experience with the commercial ELISA kit (du Pont). Cost savings would then be reflected by any reduction in the number of reaction trays required by each pooled-assay format. Estimated cost savings over individual testing costs were found both when using the pooling protocol of this study or when using the more abbreviated (hypothetical) pooling format mentioned above. Positive results obtained with the latter format (omitting individual testing of reactive pooled sera) could provide estimates for seroprevalence rates for unpooled sera by using statistical methods to estimate the expected numbers of pools with different multiple positive sera. Such estimation methods accounting for different positivity rates, multiple positivity within pools, and optimal pool size will be discussed elsewhere.

Pooling has been shown to be a viable method of performing seroprevalence testing on large populations. While allowing substantial cost savings without sacrificing either sensitivity or specificity, pooled-serum testing appears to be a reliable, efficient, and economical means for screening large numbers of serum samples from low- to moderate-risk populations for HIV antibody prevalence. However, it is not presently recommended for diagnostic testing or blood bank screening because of the theoretical loss of sensitivity.

We thank Jim Wiley and Warren Winkelstein for contributions to the study design and review of the manuscript; Annie Watson and Diane Buchanan for technical assistance; and Gail Wiggins and Maxine Hall for typing the manuscript.

LITERATURE CITED

1. Borkowsky, W., D. Paul, D. Bebenroth, K. Krasinski, T. Moore, and S. Chandwani. 1987. Human-immunodeficiency-virus infections in infants negative for anti-HIV by enzyme-linked immunosay. *Lancet* i:1168-1171.

2. **Bove, J. R., L. DePalma, and F. Weirich.** 1987. Anti-HTLV-III/LAV in pooled sera. *Clin. Chem.* **33**:308.
3. **Burkhardt, U., T. Mertens, and H. J. Eggers.** 1987. Comparison of two commercially available anti-HIV ELISAs: Abbott HTLV-III EIA and DuPont HTLV-III-ELISA. *J. Med. Virol.* **23**:217-224.
4. **Carlson, J. R., M. L. Bryant, S. H. Hinrichs, J. K. Yamamoto, N. B. Levy, J. Yee, J. Higgins, A. M. Levine, P. Holland, M. B. Gardner, and N. C. Pederson.** 1985. AIDS serology testing in low and high risk groups. *J. Am. Med. Assoc.* **253**:3405-3408.
5. **Centers for Disease Control.** 1986. Safety of therapeutic immune globulin preparations with respect to transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus infection. *Morbidity and Mortality Weekly Report*. **36**:231-232.
6. **Courouge, A.-M.** 1986. Evaluation of eight ELISA kits for the detection of anti-LAV/HTLV-III antibodies. *Lancet* **i**:1152-1153.
7. **Gallo, D., J. L. Diggs, G. R. Shell, P. J. Dailey, M. J. Hoffman, and J. L. Riggs.** 1986. Comparison of detection of antibody to the acquired immune deficiency syndrome virus by enzyme immunoassay, immunofluorescence, and Western blot methods. *J. Clin. Microbiol.* **23**:1049-1051.
8. **Gocke, D. J., J. R. K. Raska, W. Pollack, and T. Schwartz.** 1986. HTLV-III antibody in commercial immunoglobulin. *Lancet* **i**:37-38.
9. **Houn, H.-Y., A. A. Pappas, and E. M. Walker, Jr.** 1987. Status of current clinical tests for human immunodeficiency virus (HIV): applications and limitations. *Ann. Clin. Lab. Sci.* **17**:279-285.
10. **Howanitz, P. J., J. H. McBride, K. E. Kliwer, and D. O. Rodgerson.** 1986. Prevalence of antibodies to HTLV-III in quality assurance sera. *Clin. Chem.* **32**:773-777.
11. **Johnson, J. E.** 1987. Use of a commercial ELISA test for the diagnosis of infection by the AIDS virus HIV in a patient population. *Diagn. Microbiol. Infect. Dis.* **6**:267-271.
12. **Koller, U., H. Rumpold, J. Schindler, C. Schweiger, and F. Gabl.** 1987. Incidence of anti-HIV antibodies and viral antigen in standard and control sera. *J. Clin. Chem. Clin. Biochem.* **25**:705-709.
13. **Lennette, E. T., S. K. Karpatkin, and J. A. Levy.** 1987. Indirect immunofluorescence assay for antibodies to human immunodeficiency virus. *J. Clin. Microbiol.* **25**:199-202.
14. **Ozanne, G., and M. Fauvel.** 1988. Performance and reliability of five commercial enzyme-linked immunosorbent assay kits in screening for anti-human immunodeficiency virus antibody in high-risk subjects. *J. Clin. Microbiol.* **26**:1496-1500.