CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1995, p. 268–271 Vol. 2, No. 3
1071-412X/95/$04.00+0

Utility of Various Commercially Available Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kits for Use in Conjunction with Efficacy Trials of HIV-1 Vaccines

DAVID H. SCHWARTZ,[1,2]†* AVI MAZUMDAR,[2] SCOTT WINSTON,[3] AND SCOTT HARKONEN[3]

Center for Immunization Research[1] and Department of Molecular Microbiology and Immunology,[2] Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205, and Univax Biologics, Inc., Rockville, Maryland 20852[3]

Received 19 August 1994/Returned for modification 9 November 1994/Accepted 4 January 1995

There is a need for human immunodeficiency virus (HIV) screening assays which will distinguish uninfected HIV vaccine recipients from HIV-infected individuals. Commercial screening kits were used to test serum samples from low- and high-risk participants in clinical trials before and after immunization with various recombinant HIV type 1 (HIV-1) envelope glycoprotein 120 (gp120) candidate vaccines. All kits were 100% sensitive in detecting HIV infection. Both Murex Single Use Diagnostic System and United Biomedical, Inc., HIV type 1 or 2 (HIV-1/2) enzyme immunoassay (EIA) kits, which detect antibodies to HIV-1 gp41, were 98 to 100% specific when used to screen baseline or recombinant gp120-vaccinated populations as vaccine-induced antibodies to gp120 were nonreactive in these tests. The Abbott HIVAB HIV-1 EIA (lysate of whole infected cells, reactive with anti-gp120 antibodies) gave high levels of reactivity due to vaccine-induced antibodies and a high baseline rate of false positives (12 of 83) among nonvaccinated high-risk volunteers. Assays containing only gp41 and p24 solid-phase components are compatible with gp120-based vaccines but are unlikely to be useful in a similar role for vaccines containing gp160, gp41, or gp120 plus p24 antigens. Efficacy trials must be designed in concert with available diagnostic screening assays to avoid problems caused by vaccine-induced seroconversion in high-risk populations.

Several generations of licensed commercial diagnostic kits are available for the detection of human immunodeficiency virus (HIV) infection on the basis of antibody (Ab) serostatus. These kits detect the presence of immunoglobulin G antibodies to one or more immunogenic regions of HIV proteins. Licensed screening kits, used in conjunction with additional confirmatory tests such as Western blots (WBs) (immunoblots) have generally proven sufficiently sensitive and specific for their intended uses in screening blood products and donors and in monitoring high-risk populations (1, 5, 10).

More recently, however, the expansion of experimental HIV vaccine phase II trials into larger and higher-risk populations and the contemplation of even larger phase III efficacy trials of recombinant HIV type 1 (HIV-1) envelope glycoprotein 120 (rgp120) subunit vaccines involving thousands of high-risk individuals per trial arm (6) have brought to the fore the issue of compatibility between vaccine products and diagnostic assays. A number of problems may arise if HIV vaccines induce seropositivity, as measured by prevailing licensed assays, in large numbers of high-risk individuals: (i) vaccine efficacy may be more difficult to determine, as seroconversion will become an unreliable marker of infection; (ii) recruitment of volunteers may be impeded if their informed consent requires their knowledge that subsequent screening will indicate a seropositive status; and (iii) implementing long-term follow-up and secondary confirmatory assays in seropositive vaccine trial participants returning to the general high-risk population after protocol completion could cause financial, social, and logistical problems. Thus, it is essential that simple inexpensive assays which do not score uninfected high-risk vaccine recipients as positive yet which retain sensitivity and specificity for HIV infection be identified.

To date, approximately 1,000 uninfected individuals have received HIV-1 envelope-based experimental vaccines in National Institutes of Health- or industry-sponsored trials in the United States, and six vaccinees are known to have become infected as a result of intercurrent or subsequent exposure to HIV, yet there has been little coordination among manufacturers of experimental AIDS vaccines and HIV diagnostic tests. As a result, compatible combinations of current vaccines and diagnostic assays are only fortuitous and must be identified retrospectively. We present data comparing the utility of some licensed assays in the context of several rgp120 vaccine products in high- and low-risk populations.

MATERIALS AND METHODS

Subjects. Volunteers were studied in the context of three different clinical protocols involving individuals at low risk for HIV infection and one protocol involving uninfected individuals at high risk of HIV exposure. Informed consent was obtained from all patients in accordance with human experimentation guidelines of the U.S. Department of Health and Human Services and the institutional review boards of participating medical centers. Individuals were defined as being at low or high risk for acquisition of HIV on the basis of physical exams and detailed medical and sexual-behavior interviews and questionnaires as detailed previously (3, 15). Serum specimens were obtained from 13 low-risk vaccine recipients in NIAID AIDS Vaccine Evaluation Group (AVEG) protocol 006 and from 17 low-risk participants (including 1 placebo recipient) in AVEG protocol 009, which remains blinded at this time. Vaccinees in protocol 006 received three intramuscular immunizations (at 0, 1, and 8 months) with IIIB rgp120 (Genentech, Inc., South San Francisco, Calif.) followed by an additional immunization with either IIIB or MN rgp120 (Genentech) at 14 months as previously reported (3, 14, 15). Sera from participants in protocol 006 were sampled at baseline and 14, 28, and 70 days after the fourth immunization at 14 months. Protocol 009 volunteers received intramuscularly MN rgp120 with or without IIIB rgp120 at 0, 1, 6, and 12 months, and serum samples were taken at baseline and 14 to 42 days

* Corresponding author. Mailing address: Center for Immunization Research, Hampton House, Johns Hopkins School of Hygiene and Public Health, 624 North Broadway, Baltimore, MD 21205. Phone: (410) 955-1622. Fax: (410) 955-2791.
† For the NIAID AIDS Vaccine Clinical Trials Network.

after the fourth injections. An additional 114 low-risk volunteers received three 600-μg intramuscular doses of Genentech MN rgp120 as part of an unblinded 0-, 1-, and 6-month plasma donor stimulation study, protocol 2701, conducted by Univax, Inc. (Rockville, Md.). These individuals had sera sampled at baseline and 7 to 35 days after the third injection.

A total of 98 high-risk volunteers were screened for participation in AVEG protocol 201 at Johns Hopkins University School of Hygiene and Public Health. Injecting drug use was a self-declared risk factor in more than half of those screened. Of the 98 individuals screened, 43 uninfected high-risk volunteers were enrolled and received three immunizations with MN rgp120 (Genentech) or SF2 rgp120 (Chiron, Emeryville, Calif.). Sera were assayed at baseline and 14 days after the third vaccination. As the AVEG 201 trial remains blinded, sensitivity and specificity estimates include up to six placebo recipients as well as vaccinees.

Vaccines. The rgp120 vaccines derived from virus strains IIIB and MN (Genentech) or SF2 (Chiron) have been described previously (3, 4, 8, 14, 15). These vaccine products are made in Chinese hamster ovary cell line systems and are thought to assume native three-dimensional conformation with appropriate glycosylation. They were formulated for injection in alum (Genentech) or MF59 (Chiron) adjuvants.

Diagnostic assay kits. The Abbott (Abbott Park, Ill.) HIVAB HIV-1 (whole-cell lysate) enzyme immunoassay (EIA), the UBI (United Biomedical, Inc., Hauppauge, N.Y.) HIV type 1 or 2 (HIV-1/2) EIA, and the Murex (Norcross, Ga.) Single Use Diagnostic System (SUDS) Ab detection kits were used in accordance with the manufacturers' instructions. Confirmatory WB Ab detection kits were purchased from Cambridge Biotech (Worcester, Mass.) and used according to the manufacturer's instructions.

Definitions and statistics. Specificity and sensitivity were calculated for samples and individuals and rounded to two digits, as indicated in the text and Table 1. An individual volunteer was considered reactive if one or more of several serial samples scored positive, because a positive screen at any time during or after immunization would require further testing in the setting of a field trial.

Because of the rgp120 contents of the vaccines used, routine criteria for positive and indeterminate WB results were not used for postvaccination samples. Instead, samples were considered to be from HIV-infected donors if they scored positive for WB bands gp160, gp120, gp41, p24, and p17 plus one or more of the bands p66, p55, p51, and p31. Volunteers testing positive for HIV infection by WB did not receive subsequent vaccinations. Because baseline and multiple serial samples were available for analysis, WB patterns fitting the standard technical definition of "indeterminate" could always be shown to be from uninfected volunteers. That is, for all indeterminate postvaccination WBs, non-envelope band reactivities were present at baseline and/or absent at a subsequent time. Therefore, such WB patterns were defined as negative for the purposes of this study. All volunteers were tested by WB for serostatus at baseline, after final injections, and in most cases at the time of each screening assay. Volunteers testing negative (as defined above) by WB were assumed to have been HIV seronegative at all times prior to the WB. Using these criteria, we applied the following definitions: assay specificity = number negative by assay/[(number negative by assay and WB) + (number positive by assay and negative by WB)] and assay sensitivity = number positive by assay/[(number positive by assay and WB) + (number negative by assay and positive by WB)]. Specificities were tested for significant differences by the two-sample Z test for proportions with equal or unequal denominators.

RESULTS

Screening high-risk individuals. WBs confirmed HIV infection in 15 of 98 high-risk individuals tested (15%) (all bands present). The Abbott HIV-1, UBI HIV-1/2, and Murex Ab screening tests were 100% sensitive, detecting all true HIV-positive samples. However, the Abbott HIV-1 test reported an additional 12 uninfected individuals' samples as positive (specificity = 86%). The UBI HIV-1/2 and Murex SUDS kits each gave two false-positive results (specificity = 98%).

Screening for vaccine-induced seroconversion of low-risk rgp120-vaccinated individuals. The 13 participants in protocol 006 were tested 14, 28, and 70 days after four immunizations with IIIB rgp120 alone or with IIIB and MN rgp120 (Table 1). A total of 39 serum samples were tested by Abbott HIV-1 EIA, with 12 positive results (specificity = 69%) derived from 8 of 13 volunteers (specificity for participants = 39%). No samples scored positive 70 days after vaccination. The same 39 samples tested with UBI HIV-1/2 EIA and Murex SUDS kits gave no positive results, yielding a significantly better specificity of 100% ($P < 0.0001$). WB testing of all 39 samples showed no banding patterns consistent with HIV infection, although in several cases the expected positive bands corresponding to vaccine-induced gp120 reactivity were present.

TABLE 1. Specificities of three commercial assays for sera from uninfected (WB-negative) recipients of more than three immunizations with HIV-1 rgp120 vaccines

Protocol[a]		No. of samples (no. of volunteers)[b]			Specificity (%)
		Negative	Positive	Total	
006*	Abbott HIV-1	27 (5)	12 (8)	39 (13)	69 (39)
	UBI HIV-1/2	39 (13)	0 (0)	39 (13)	100 (100)
	Murex SUDS	37 (13)	0 (0)	37 (13)	100 (100)
009**	Abbott HIV-1	36 (13)	4 (4)	40 (17)	90 (77)
	UBI HIV-1/2	40 (17)	0 (0)	40 (17)	100 (100)
	Murex SUDS	40 (17)	0 (0)	40 (17)	100 (100)
201*	Abbott HIV-1	34 (34)	9 (9)	43 (43)	79 (79)
	UBI HIV-1/2	42 (42)	1 (1)	43 (43)	98 (98)
	Murex SUDS	43 (43)	0 (0)	43 (43)	100 (100)
Composite*	Abbott HIV-1	97 (52)	25 (21)	122 (73)	80 (71)
	UBI HIV-1/2	121 (72)	1 (1)	122 (73)	99 (99)
	Murex SUDS	122 (73)	0 (0)	122 (73)	100 (100)

[a] *, $P < 0.0001$ for Abbott HIV-1 EIA versus UBI HIV-1/2 EIA or Murex SUDS; **, $P < 0.05$ for Abbott HIV-1 EIA versus UBI HIV-1/2 EIA or Murex SUDS.

[b] Volunteer considered positive if one or more sequential samples scored positive.

The 17 participants (including one placebo recipient) in protocol 009 were tested 14 to 42 days after four immunizations with MN rgp120 or MN rgp120 plus IIIB rgp120. Four of the 17 individuals were the source of 4 of 40 samples testing positive by Abbott HIV-1 EIA without evidence of HIV infection on WB (90% specificity). None scored positive with the Murex SUDS or the UBI HIV-1/2 EIA (100% specificity).

An unselected subset of 58 protocol 2701 participants provided 243 samples 7 to 35 days after three immunizations for evaluation by Abbott HIV-1 EIA. Of these samples, 29 (from 14 individuals) tested positive (sample specificity = 88%; individual volunteer specificity = 76%). The same 29 serum specimens scored negative by UBI HIV-1/2 and Murex SUDS assays and by WB corrected for envelope and preexisting p24 bands ($P < 0.0001$).

Screening for vaccine-induced seroconversion of high-risk rgp120-vaccinated individuals. As shown in Table 1, 43 high-risk participants in AVEG protocol 201 were assessed 14 days after receiving three injections of either MN or SF2 rgp120 or a placebo ($n \leq 6$). Nine samples tested by Abbott HIV-1 EIA scored positive (specificity = 79%). All of these samples were negative by SUDS assay (100% specificity), while one scored positive in the UBI HIV-1/2 EIA (specificity = 98%).

Table 1 compares assay specificities, after three or more rgp120 immunizations, for 122 serum samples from 73 individuals participating in experimental vaccine protocols involving high- or low-risk volunteers. Specificities of Abbott HIV-1, UBI HIV-1/2, and Murex HIV-1 SUDS assays calculated for total samples were 80, 99, and 100%, respectively. The corresponding specificities calculated for individual participants were 71, 99, and 100%. With either total sample- or participant-based calculations, the specificity of the Abbott HIV-1 assay is statistically less than that of the other two kits ($P < 0.0001$).

DISCUSSION

To be useful in large vaccine efficacy field trials involving thousands of high-risk participants, any HIV screening test should have a false-positive rate that is much lower than the incidence of infection (targeted at ~5% per year for most proposed studies). In this context, "false"-positive results must include "true" reactivities due to vaccine-induced HIV-specific Abs. Our results indicate that, in the North American populations studied, both the UBI HIV-1/2 and the Murex SUDS tests have acceptable sensitivities and specificities for use in vaccine trials employing MN, IIIB, or SF2 rgp120 vaccine products. As both tests rely primarily on reactivity of Abs with gp41 solid-phase epitopes, they would not be expected to be useful in trials of vaccines containing full-length intact gp160 or gp41.

The Abbott whole-cell lysate HIVAB HIV-1 EIA does not appear to be useful for rgp120-based vaccine trials, presumably because of the inclusion of significant amounts of gp120 envelope protein in the Ab capture solid-phase component. Our data also indicate an unacceptably high number of false-positive results among uninfected nonvaccinated populations composed largely of injecting drug users, presumably due to high levels of alloantibodies. This low specificity has not been seen with other risk groups, underscoring the need to pretest assays with panels of sera from the population for which field trials are planned. It may not be sufficient to rely on specificity data obtained for groups with markedly different risk factors, geographic distributions, or genetic backgrounds.

No sera gave WB patterns that were still considered indeterminate after correction for expected envelope protein (gp120 and gp160) and baseline plus follow-up reactivities. However, this correction could be performed only by an individual (i) familiar with the expected pattern of vaccine-induced reactivity and (ii) having access to baseline and follow-up WB results. Many of the sera tested showed weak or no reactivity with gp120 on WB, indicating relatively low levels of envelope-specific binding. In most cases, EIA and/or WB gp120 reactivity occurred within 5 weeks of booster injections. More-potent vaccines inducing higher Ab levels for more extended periods could result in even greater numbers of vaccine-induced "false" positives in assay systems incorporating gp120 components. Similarly, induction of high levels of Abs by gp160- or gp41-based vaccines would be expected to markedly reduce the observed specificity of the Murex SUDS and UBI HIV-1/2 assays.

For now, empiric testing of specific combinations of proposed vaccines with various screening assays in defined populations targeted for trials is necessary. In the future, issues of diagnosis and vaccine-induced serostatus should be prospectively addressed by the vaccine development and evaluation community. As new generations of vaccines become more complex and inclusive with respect to numbers of HIV epitopes included, it will become increasingly difficult to devise simple, sensitive, and inexpensive screening assays to discriminate between vaccine-induced seroconversion and infection.

Existing confirmatory assays are unlikely to be suitable solutions for this problem. In particular, WBs containing multiple vaccine-induced bands are not readily distinguished from those showing early seroconversion due to infection, especially in populations with a high proportion of preexisting reactivity to p17 and p24 bands (2, 7, 12) and in the face of vaccine-induced Ab responses. Cheap and specific PCR-based assays for direct detection of infection are not yet available and may not be sufficiently sensitive in the setting of preexisting vaccine-induced immune responses leading to reduced viral loads (9, 11, 13). Viral culture as a screening test is not feasible.

At a practical level, the Murex SUDS has advantages of being usable under field conditions without equipment, with a turnaround time of minutes. However, the cost of this assay (~$10.00 per test versus ~$1.75 for UBI HIV-1/2) may have to be reduced considerably for phase III trials. Ultimately, it will be self-defeating if a suitably inexpensive vaccine (estimated by some at ≤$1.00 per dose to be affordable in many countries) can be assessed only in the context of long-term frequent screening with far more costly diagnostic assays, such as WBs costing >$10 per test, even when purchased in bulk by the World Health Organization (16). These hidden costs have been omitted from previous economic analyses of vaccine trials. Furthermore, if an appropriate diagnostic assay is identified, it may be necessary to make it the predominant or exclusive licensed assay for use among the larger populations from which the vaccinated volunteers are drawn. Otherwise, vaccinees being tested in settings other than the clinical trial may face adverse consequences of positive test results, even with documentation of trial participation.

ACKNOWLEDGMENTS

We thank UBI and Murex for donating diagnostic kits.

This work was supported by NIAID contracts NO1-AI-05061, NO1-AI-0562, NO1-AI-05064, NO1-AI-05065, and NO1-AI-82500.

REFERENCES

1. **Alter, J. H., J. S. Epstein, S. G. Swenson, M. J. VanRaden, J. W. Ward, R. A. Kaslow, J. E. Menitove, H. G. Klein, S. G. Sandler, M. H. Sayers, I. K. Hewlett, A. I. Chernoff, and HIV-Antigen Study Group.** 1990. Prevalence of human immunodeficiency virus type 1 p24 antigen in U.S. donors—and assessment of the efficacy of testing in donor screening. N. Engl. J. Med. **323:**1312–1317.
2. **Behets, F., A. Disasi, R. W. Ryder, K. Bishagara, P. Piot, M. Kashamuka, M. Kamenga, N. Nzila, M. Laga, G. Vercauteren, V. Batter, C. Brown, and T. Quinn.** 1991. Comparison of five commercial enzyme-linked immunosorbent assays and Western immunoblotting for human immunodeficiency virus antibody detection in serum samples from Central Africa. J. Clin. Microbiol. **29:**2280–2284.
3. **Belshe, R., M. Keefer, B. Graham, T. Matthews, T. Twaddell, and P. Fast.** 1993. Safety and immunogenicity of HIV-1 (MN or combination MN/IIIB) rgp120 vaccines in low risk volunteers, abstr. WS-B27-1. *In* IXth International Conference on AIDS. World Health Organization, Geneva.
4. **Berman, P. W., T. J. Gregory, L. Riddle, G. R. Nakamura, M. A. Champe, J. P. Porter, F. M. Wurm, R. D. Hershberg, E. K. Cobb, and J. W. Eichberg.** 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature (London) **345:** 622–625.
5. **Busch, M. P., P. E. Taylor, B. A. Lenes, S. H. Cleinman, M. Stuart, C. E. Stevens, P. A. Tomasulo, J. P. Allain, C. G. Hollingsworth, and J. W. Mosley.** 1990. Screening of selected male blood donors for p24 antigen of human immunodeficiency virus type 1. N. Engl. J. Med. **323:**1308–1312.
6. **Doepel, L. K.** 1994. NIAID committed to efficacy trials with appropriate candidate HIV vaccines, p. 3–11. *In* M. E. Glick (ed.), NIAID AIDS Agenda, winter issue.
7. **Genesca, J., J. Shih, B. W. Jett, I. K. Hewlett, J. S. Epstein, and H. J. Altr.** 1989. What do Western blot indeterminate patterns for human immunodeficiency virus mean in EIA-negative blood donors? Lancet **ii:**1023–1025.
8. **Graham, B., M. Keefer, and J. McElrath.** 1993. Phase I trial of native HIV-1 SF2 rgp120 candidate vaccine, abstr. PO-A29-0692. *In* IXth International Conference on AIDS. World Health Organization, Geneva.
9. **Johnson, B. K., G. A. Stone, M. S. Godec, D. M. Asher, D. C. Gajdusek, and C. J. Gibbs, Jr.** 1993. Long-term observations of human immunodeficiency virus-infected chimpanzees. AIDS Res. Hum. Retroviruses **9:**375–378.
10. **Johnson, J. E.** 1992. Detection of human immunodeficiency virus type 1 antibody by using commercially available whole-cell viral lysate, synthetic peptide, and recombinant protein enzyme immunoassay systems. J. Clin. Microbiol. **30:**216–218.
11. **Lee, T. H., Z. el-Ahmad, M. Reis, M. Adams, E. A. Donegan, T. R. O'Brien, A. R. Moss, and M. P. Busch.** 1991. Absence of HIV-1 DNA in high-risk seronegative individuals using high-input polymerase chain reaction. AIDS **5:**1201–1207.
12. **Midthun, K., L. Garrison, M. L. Clements, B. Fernie, H. Farzedagen, and T. Quinn.** 1990. Frequency of indeterminate Western blot tests in healthy

adults at low risk for human immunodeficiency virus infection. J. Infect. Dis. **162:**1379–1382.

13. **Schechter, M. T., P. W. Neumann, M. S. Weaver, J. S. Montaner, S. A. Cassol, T. N. Le, K. J. Craib, and M. V. O'Shaughnessy.** 1991. Low HIV-1 proviral DNA burden detected by negative polymerase chain reaction in seropositive individuals correlates with slower disease progression. AIDS **5:**373–379.
14. **Schwartz, D., M. L. Clements, and R. Belshe.** 1993. Antibody responses to neutralizing domains of gp120 in HIV (−) vaccinees boosted with homologous or heterologous rgp120, abstr. PO-A29-0690. *In* IXth International Conference on AIDS. World Health Organization, Geneva.
15. **Schwartz, D. H., G. Gorse, M. L. Clements, R. Belshe, A. Izu, A. M. Duliege, P. Berman, T. Twaddell, D. Stablein, R. Sposto, R. Siliciano, and T. Matthews.** 1993. Safety and immunogenicity of a baculovirus derived HIV-1_{IIIB} rgp120 subunit vaccine. Lancet **342:**69–73.
16. **Tamashiro, H., W. Maskill, J. Emmanual, A. Fauquex, P. Sato, and D. Heymann.** 1993. Reducing the cost of HIV antibody testing. Lancet **342:**87–90.