Human Immunodeficiency Virus (HIV)
Seropositivity among Uninfected HIV Vaccine Recipients

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Since 1987, >10,000 individuals worldwide have received immunizations with human immunodeficiency virus (HIV) preventive vaccine constructs. Many constructs elicit antibodies detected by standard serologic tests (enzyme immunoassays, rapid tests, and Western blots) and result in vaccine recipients’ serum being identified as reactive and indicative of HIV infection. To determine the frequency of vaccine-induced HIV antibody among uninfected HIV vaccine trial participants and to identify factors associated with these results, serum samples from HIV-uninfected participants from selected United States phase I/II HIV-1 vaccine trials were tested with 6 serologic screening tests. Reactive specimens were tested by use of Western blot. Overall, 490 serum specimens from 461 vaccine recipients were tested; 100 (20.4%) reacted on at least 1 serologic test, and 65 (13%) were determined to be positive by Western blot. Canarypox or vaccinia vaccine recipients’ serum with or without HIV envelope glycoprotein (gp120 or gp160) boosts accounted for all positive Western blot results; no positive Western blot results were obtained from gp120 subunit recipients. The potential for vaccine recipients being misclassified as HIV infected increased with vaccine complexity.

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All vaccine trial participants provided written informed consent for human immunodeficiency virus testing and trial participation, and human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this research.

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administered, duration of time since the last immunization, and type of serologic test used [4–8]. Clarification of these seropositive results among vaccine recipients has resulted in the use of more complicated laboratory techniques, such as RNA detection, DNA polymerase chain reaction (PCR) analysis, and viral culture. The use of such tests and complicated testing algorithms in the posttrial setting can be labor intensive and costly [9, 10].

In the past, the number of persons participating in clinical vaccine trials has been small, and difficulties distinguishing between antibodies produced by HIV infection and vaccine-induced antibodies by standard serologic tests were limited to individual clinical sites with the capability to conduct additional laboratory testing. However, with the advent of the first phase III HIV vaccine efficacy trials in North America [2] and Thailand [3] (bivalent rgp120 products, AIDSVAX B/B and AIDS-VAX B/E; VaxGen), an additional 4800 participants have received an HIV vaccine. Future large-scale vaccine trials will greatly increase the number of vaccine recipients [11, 12]. To determine the frequency of vaccine-induced HIV antibody results or “false-positive results” among uninfected HIV-1 vaccine recipients and to identify factors associated with these results, we tested serum specimens collected from uninfected HIV vaccine recipients who participated in select US phase I and II clinical trials from 1993 through 1999.

SUBJECTS, MATERIALS, AND METHODS

Subjects. From February 1988 to May 2000, the National Institutes of Health–sponsored AIDS Vaccine Evaluation Group (AVEG) conducted >50 phase I HIV-1 vaccine trials (comprising 35 linked studies) in the United States [13]. Six of these phase I trials involved 562 participants and were conducted between 1993 and 1998. In addition, from 1997 through 1999, VaxGen (Brisbane, CA) conducted a HIV-1 phase I/II vaccine trial among 120 US participants. All 682 vaccine participants were healthy individuals, aged 18–60 years, generally assessed to be at low risk for HIV exposure (although 1 AVEG trial enrolled persons at higher risk for HIV infection through sexual or injection drug use exposure [14]), enrolled at 8 US clinical trial sites in major metropolitan areas, and negative for HIV-1 by ELISA at the time of enrollment. In 4 of the 6 AVEG trials, participants also were required to be negative for HIV-1 by Western blot upon enrollment.

Vaccine constructs and study regimens. Three types of HIV-1 vaccine constructs were evaluated in the 6 AVEG trials that are described here: (1) the recombinant protein subunit rgp120 SF-2 combined with MF59, an oil-in-water adjuvant (Chiron); (2) TBC-3B (a live, recombinant vaccinia virus expressing the env and gag/pol genes of HIV-1 strain IIIb, grown in African green monkey kidney cells; Therion Biologics) administered through 1 of 3 routes (scarification, intradermal, and subcutaneous) followed by boosts of 300 µg rgp120 MN (VaxGen); and (3) 5 ALVAC recombinant canarypox constructs (vCP125 [gp160], vCP205 [gp120, gp41, gag, and protease], vCP300 and vCP1433 [vCP205 with additional sequences from pol and nef], and vCP1452 [vCP205, with additional sequences from pol and nef, and 2 vaccinia virus coding sequences]) (Pasteur Mérieux/Connaught [now Aventis Pasteur]), alone or with rgp120 SF-2 priming or boosts or rgp160MN/LAI boosts (Pasteur Mérieux/Connaught). Control preparations included the following: an ALVAC recombinant canarypox expressing the rabies glycoprotein (vCP65) with or without rgp120 SF-2 priming or boosts and the formerly licensed vaccinia vaccine (DryVax; Wyeth Laboratories). Studies included 4–6 immunizations administered over 3–12 months, and serum specimens were collected from participants before and after each vaccination, to evaluate the safety, toxicity, and immunogenicity of the vaccine constructs. During the AVEG trials, HIV infection was excluded from these participants’ specimens by use of algorithms that included serologic and nucleic acid–based tests and culture. Details of the study designs have been reported elsewhere [13].

The VaxGen phase I/II vaccine trial (VAX 002) evaluated a recombinant protein subunit vaccine, rgp120, in 2 bivalent formulations: AIDSVAX B/B containing MN rgp120/HIV-1 and GNE8 rgp120/HIV-1, and AIDSVAX B/E containing MN rgp120/HIV-1 and A244 rgp120/HIV-1 in an aluminum hydroxide adjuvant. Thirty participants received 300 µg of each antigen (B and E), and 90 participants were randomly assigned to 1 of 3 different B/E study groups (100, 300, or 600 µg). There was no placebo group. All participants received 4 doses over 12 months. Serum specimens were collected from participants at enrollment, before and 2 weeks after each immunization, and at study conclusion [15, 16].

Specimen selection. Serum samples selected from a subset of uninfected HIV vaccine trial participants were forwarded by the AVEG repository site and VaxGen to the Centers for Disease Control and Prevention (CDC) for analysis. AVEG specimens forwarded to CDC included serum samples from gp120 SF-2 and canarypox vaccine recipients collected 2 weeks after the last vaccination, as well as serum samples from vaccinia vaccine recipients collected 2 weeks after the second and last vaccinations. VaxGen specimens forwarded to CDC included serum samples from gp120 vaccine recipients collected 2 weeks after the third vaccination. Specimens were convenience sampled and selected within treatment groups and protocols based on sample availability. Specimens from AVEG groups that were likely to cross-react were overrepresented in the sample selection. In particular, a number of AVEG specimens included gp160 boosts and, therefore, the immunodominant domain of gp41 and were not representative of all AVEG vaccine trials.

Laboratory testing. Specimens were tested by use of 6 se-
rologic tests that encompass a variety of antigens. Three US Food and Drug Administration (FDA)–licensed EIA tests (HIV-1/HIV-2 Peptide EIA and rLAV EIA [Genetic Systems] and OTC Vironostika HIV-1 MicroELISA System [Organon Teknika]), 1 non–FDA-licensed EIA test (Biochem SELECT HIV [Biochem ImmunoSystems]), and 1 non–FDA-licensed rapid test (Sero-Strip [Saliva Diagnostics]) were used for all serum specimens tested. The FDA-licensed Abbott 3A11 EIA (Abbott Laboratories) was added for rgp120 B/B and B/E serum samples, because it was the only EIA to be used in the AIDSvAX phase III trial HIV testing algorithm. Antibody detection kits were used according to the manufacturers’ instructions. Supplemental testing by Western blot (Cambridge Biotech) was performed only if a specimen reacted to at least 1 HIV serologic screening test. HIV seropositivity was defined as a reactive screening test and the presence of any 2 of the bands gp41, gp120/160, or p24 on Western blot (criteria used by the Association of Public Health Laboratories/ CDC) [17]. A negative Western blot would be the absence of any bands, and an indeterminate Western blot would be the presence of ≥1 bands that do not meet the criteria necessary for a positive result.

RESULTS

In total, 490 serum specimens collected from 461 uninfected HIV vaccine recipients who participated in 34 groups of 7 protocols were tested. Single specimens were available from 432 persons immunized with 1 of 5 different rgp120 subunit vaccine constructs (n = 118; table 1) or from ALVAC canarypox vaccine recipients (n = 314; table 2). Two samples each were available from 29 persons immunized with a vaccinia construct (table 3). Of the 490 specimens collected, 100 (20.4%) reacted on at least 1 serologic screening test. Western blots performed on these 100 specimens produced 65 positive results, 31 indeterminate results, and 4 negative results.

Subunit vaccine constructs. One hundred eighteen serum samples from vaccine recipients who were administered 1 of 5 different rgp120 subunit vaccine constructs were tested (table 1). Of these 118 participants, 108 (91.5%) received 1 of 2 different bivalent rgp120 formulations, and 10 received a SF-2 rgp120 construct. Eighteen (15.2%) serum specimens reacted on EIA (14 specimens) or the rapid assay (4 specimens). Western blots were performed on all 18 reactive specimens, all of which yielded indeterminate results. Western blot patterns included gp120 alone (13 specimens), gp120 and p51 (2 specimens), or gp120, p66, and p51 (3 specimens).

The Genetic Systems rLAV EIA most commonly yielded a positive antibody result. This EIA was positive for 13 (11.0%) specimens. In contrast, none of the other FDA-licensed kits detected antibody, and the 2 non–FDA-licensed tests detected antibody in 5 (4%) specimens. Serum samples from vaccine recipients administered large (300 or 600 μg) doses of the B/B rgp120 construct were more likely (23.5%) to yield reactive serologic screening test results, versus vaccine recipients receiving 100-μg B/B, 300-μg B/E, and 50-μg SF-2 vaccines (9.0%) (P < .05).

Canarypox constructs alone or with recombinant envelope subunits. The majority of specimens tested, 314 (64.1%) of 490, were collected from ALVAC canarypox vaccine recipients (table 2). Of these 314 specimens, 65 (20.7%) were antibody positive on at least 1 EIA or rapid assay; 4 (6%) were from participants receiving the vCP65 control vaccine. Western blot results for the 65 serologically reactive specimens were as follows:

**Table 1. Human immunodeficiency virus (HIV) screening test results on serum specimens from HIV-uninfected gp120 subunit vaccine recipients, by serologic test.**

<table>
<thead>
<tr>
<th>gp120 Constructs, dose</th>
<th>No. of samples</th>
<th>Serologic screening testa</th>
<th>Positive specimens, no. (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FDA licensed</td>
<td>Non–FDA licensed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abbott 3A11 EIA</td>
<td>HIV-1/ HIV-2 Peptide EIA</td>
</tr>
<tr>
<td>gp120 B/B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μg</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300 μg</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>600 μg</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp120 B/E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 μg</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp120 SF-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μg</td>
<td>10</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** ND, not done.

a US Food and Drug Administration (FDA)–licensed tests: Abbott 3A11 EIA (Abbott Laboratories), HIV-1/HIV-2 Peptide EIA and rLAV EIA (Genetic Systems), and Vironostika HIV-1 MicroELISA System (Organon Teknika); Non–FDA-licensed tests: Biochem SELECT HIV (Biochem ImmunoSystems) and Sero-Strip (Saliva Diagnostics).

b Reflects no. of specimens seropositive on at least 1 serologic test among the total no. tested.
Table 2. Human immunodeficiency virus (HIV) screening and confirmatory test results on serum specimens from HIV-uninfected canarypox vaccine recipients, by serologic test

<table>
<thead>
<tr>
<th>Canarypox construct, ± rgp120 or rgp160 subunit</th>
<th>No. of samples</th>
<th>FDA licensed</th>
<th>Non-FDA licensed</th>
<th>EIA-positive specimens, no. (%)</th>
<th>Western blot results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HIV-1/HIV-2 Peptide EIA</td>
<td>rLAV EIA</td>
<td>Vironostika HIV-1</td>
<td>SELECT HIV</td>
</tr>
<tr>
<td>vCP65 (control) ± adjuvant, gp120 repertoire</td>
<td>49</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>vCP65 (control) first 2 doses + gp120 only</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>vCP125</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>vCP300</td>
<td>33</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>vCP125 first 2 doses + gp120 only last 2 doses</td>
<td>30</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>vCP205 first 4 doses + gp120 only for the last 2 doses</td>
<td>22</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>vCP300 first 2 doses + gp120 only last 2 doses</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>vCP300 + gp120</td>
<td>35</td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>vCP300 + gp160</td>
<td>17</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>vCP300 all doses + gp120 last 2 doses</td>
<td>32</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>vCP205 all doses + gp160 last 2 doses</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>vCP1433 all doses + gp160 last 2 doses</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>vCP1452 all doses + gp160 last 2 doses</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>314</td>
<td>26</td>
<td>56</td>
<td>34</td>
<td>23</td>
</tr>
</tbody>
</table>

a US Food and Drug Administration (FDA)-licensed tests: HIV-1/HIV-2 Peptide EIA and rLAV EIA (Genetic Systems) and Vironostika HIV-1 MicroELISA System (Organon Teknika); Non–FDA-licensed tests: Biochem SELECT (Biochem Immunosystems) and Sero-Strip (Saliva Diagnostics).

b Reflects no. of specimens seropositive on at least 1 serologic test among the total no. tested.

4 (6.1%) negative, 12 (18.5%) indeterminate, and 49 (75.4%) positive. None of the control specimens was determined to be positive by Western blot. Canarypox vaccine constructs with the rgp160 boosts had the highest percentage of reactive specimens; 24 (92.3%) of 26 specimens were reactive on at least 1 serologic screening test, and all 24 specimens were determined to be positive by Western blot.

All EIA kits were HIV antibody positive for at least 1 specimen, and 33 (56.9%) of 58 EIA-reactive canarypox vaccine construct specimens (vCP control specimens with or without gp120 excluded) reacted on ≥2 test kits. Overall, the Genetic Systems rLAV EIA yielded the largest number of positive results. It identified antibody in 21.2% of these canarypox vaccine construct recipient serum samples, compared with the other 3 EIA kits that identified antibody in 13.1% (OTC Vironostika), 10.2% (Genetic Systems HIV-1/2), and 9.0% (Biochem SELECT) of samples. EIA test results among the canarypox vaccine recipients with the rgp160 boosts were more consistently positive. Of these 26 specimens, 23 (88.4%) reacted in Genetic Systems HIV-1/2, 24 (92.3%) in Genetic Systems rLAV, 23 (88.4%) in OTC Vironostika, and 22 (84.6%) in Biochem SELECT.

**Vaccinia constructs alone or with rgp120 subunits.** Fifty-eight serum samples were collected at 2 time points from 29 persons who received a vaccinia construct (TBC-3B or the DryVax placebo; table 3). Overall, 17 (29.3%) of the 58 serum specimens reacted on at least 1 serologic test. All 17 reactive specimens were from TBC-3B vaccinia vaccine recipients (17 [35.4%] of 48); there were no reactive specimens among the DryVax placebo recipients’ serum samples. Serum samples from vaccine recipients who only received intradermal vaccinations of TBC-3B had the largest proportion of reactive specimens, 6 (67%) as compared with recipients administered TBC-3B by scarification, 0%, or subcutaneously. 43%. However, serum samples from recipients receiving TBC-3B with gp120 boosts demonstrated a larger proportion of reactive specimens after subcutaneous administration (57%), compared with vaccine scarification and intradermal administration (25% and 22%, respectively). In addition, although specimens were collected...
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Table 3. Human immunodeficiency virus (HIV) screening and confirmatory test results in serum specimens from HIV-uninfected vaccinia vaccine recipients, by serologic test.

<table>
<thead>
<tr>
<th>Vaccinia construct ± gp120</th>
<th>No. of samples</th>
<th>FDA licensed</th>
<th>Non-FDA licensed</th>
<th>EIA-positive specimens, no. (%)</th>
<th>Western blot results</th>
</tr>
</thead>
<tbody>
<tr>
<td>DryVax (control)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TBC-3B</td>
<td>24</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>TBC-3B first 2 doses + gp120 only for the last 2 doses</td>
<td>24</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>1</td>
<td>15</td>
<td>2</td>
<td>17 (29.3)</td>
</tr>
</tbody>
</table>

* FDA-licensed tests: HIV-1/HIV-2 Peptide EIA and rLAV EIA (Genetic Systems) and Vironostika HIV-1 MicroELISA System (Organon Teknika); Non-FDA-licensed tests: Biochem SELECT (Biochem Immunosystems) and Sero-Strip (Saliva Diagnostics).

b Reflects no. of specimens seropositive on at least 1 serologic test among the total no. tested.

c TBC-3B administered by 1 of 3 routes (scarification, intradermal, and subcutaneous).

from each individual at 2 separate time points, individuals’ test results did not remain consistent over time. Of the 9 reactive vaccinia specimens collected after the second TBC-3B vaccination, only 3 (33%) specimens remained reactive at the next time point after gp120 vaccination, and another 3 recipients’ samples that had previously been nonreactive were now determined to be reactive. Western blot testing among the 17 reactive specimens yielded 16 (94.1%) positive results; the remaining specimen had only a gp120 band present and was indeterminate.

Each type of EIA kit detected the presence of vaccine-induced HIV antibody in ≥1 of the 17 reactive specimens. The Genetic Systems rLAV EIA produced the largest number of reactive results; it identified HIV antibody in 31.2% of TBC-3B recipient specimens, compared with 12.5% (OTC Vironostika), 4.2% (Biochem SELECT), and 2.1% (Genetic Systems HIV-1/2) of specimens with the other 3 EIA kits.

**DISCUSSION**

These data indicate that a substantial proportion of HIV vaccine recipients may have seropositive screening test results after vaccination. In our study, 20% of specimens from selected vaccine recipients reacted on at least 1 of 6 HIV-1 screening tests and 13% were confirmed to be positive by Western blot (if control recipients were excluded, these percentages would increase to 22.4% and 15%, respectively). EIA-reactive results among serum specimens ranged from 15.2% for recombinant protein subunits, to 20.7% for canarypox constructs (including the 4 EIA-reactive vCP65 controls), and to 29.3% for vaccinia constructs. This is consistent with the 8%–30% rate obtained by the AVEG in numerous trials using the Abbott HIV-1/2 EIA (T.G.E., unpublished data). No positive Western blot patterns were detected in serum samples from gp120 subunit recipients; all 65 positive patterns were obtained from serum samples of participants administered canarypox or vaccinia constructs with or without recombinant subunit proteins.

Vaccine-induced EIA reactivity depends on the components used in both vaccine constructs and test kits. Many EIA-positive serum specimens reacted on only 1 EIA, Genetic Systems rLAV, which is a highly sensitive EIA that uses a whole viral lysate with enriched envelope protein as antigens, compared with OTC Vironostika (viral lysate only) and the Genetic Systems HIV-1/2 (synthetic peptides from env and pol). Western blot testing of EIA-reactive gp120 vaccine recipients, who currently constitute the largest group of vaccine recipients, yielded only indeterminate results, which indicates that, although these vaccine recipients may be requested to undergo repeat HIV testing, they have a low likelihood of being misclassified as HIV-1 infected. In contrast, vaccine recipients administered more complex constructs or combinations of vaccines (i.e., vaccinia or canarypox vectors with gp160 subunit boosts) had a higher overall percentage of reactive screening test results due to the presence of gp41 in both test kits and vaccine constructs. These constructs were less dependent on the type of screening test and had a higher rate of positive Western blot tests. These findings indicate that although current screening EIA kits containing synthetic peptide antigens work satisfactorily for the majority of vaccine trial recipients who only received gp120 recombinant protein subunit constructs, their performance is severely limited when testing more immunogenic constructs containing gp160 or the Avery peptide (immunodominant region of gp41). Thus, the potential for vaccine recipients being misidentified as being HIV infected increased with the administration of more immunologically complex or combination vaccines.

A 13% HIV seropositivity rate among selected uninfected vaccine recipients has at least 3 important implications. First, a high rate of vaccine-induced seropositivity may result in serious consequences for vaccine recipients mistakenly identified...
as HIV infected based on their serologic test results. These individuals may be subjected to unnecessary medical therapy if confirmatory testing is not performed or does not accurately represent their true infection status. They also could be exposed to social harms, such as the denial of employment, travel opportunities, and health or life insurance, and experience disruption within their personal relationships. Several such situations occurred in the mid-1990s among phase I/II trial participants [5, 6]. One participant faced dismissal from the armed forces, a second was denied health insurance [5], and 6 were unable to donate blood [6] after receipt of a positive HIV test result obtained from testing performed outside the trial setting. Study personnel intervened in the first 2 situations to clarify the participants’ HIV infection status and to avoid the potentially damaging outcomes of being misidentified as HIV infected. However, issues around blood donation are not so easily resolved given the need to protect the public’s blood supply. As more people participate in large-scale trials that use increasingly more potent vaccine constructs, other social harms may arise and become more common and more complex to resolve. Future trials may require study sponsors to provide long-term free confirmatory HIV testing for study participants with vaccine-induced antibodies and assistance with social harm resolution.

The second implication relates to HIV surveillance and program planning. In the past, US public health officials relied on AIDS case reporting to monitor the epidemic. However, over the last few years in an effort to improve disease surveillance and to develop and evaluate strategies for HIV prevention programs, public health officials have placed increasing emphasis on HIV testing and reporting. Future HIV-1 efficacy trial participants recruited from high-risk populations could have both a high probability of having vaccine-induced antibody from their trial participation and being among those population groups targeted for HIV testing, thereby artificially increasing surveillance estimates and distorting intervention outcomes. Current HIV counseling, testing, and referral guidelines advise HIV vaccine trial participants with positive test results to contact or return to their trial site or an associated site for HIV counseling and evaluation [18].

Third, informing potential participants about the significant possibility of having reactive serologic tests could have implications for the conduct of future efficacy trials. This information could deter potential volunteers from participating because of concerns about the implications of a false-positive test [19, 20]. In 1 study of factors affecting homosexual men’s willingness to participate in future HIV vaccine efficacy trials, 37% of potential participants responded that the presence of a vaccine-induced seropositive test result would adversely influence their vaccine trial participation [19], because, although vaccine clinical trial sites offer additional and confirmatory HIV-1 testing for participants, individuals may be tested at outside sites for nontrial reasons [21]. Past studies have indicated that as many as 22%–26% of phase I/II HIV vaccine trial participants reported being tested for HIV at locations other than their study site [22, 23]. In addition, the strong possibility of having a reactive screening test may tempt 13%–15% of trial participants to seek outside testing to determine whether they received vaccine or placebo and, therefore, “unblind” their immunization status while the study is ongoing [24, 25]. Unblinding may result in participants altering their risk behavior based on perceived vaccine or placebo receipt and thereby invalidate study findings, as well as place them at risk for HIV infection.

Although investigators of phase I/II HIV vaccine trials have been successful at distinguishing true infection from vaccine-induced antibodies, future vaccine constructs (i.e., DNA-based, envelope-CD4 receptor complexes, and others) potentially heading toward small clinical and large phase III efficacy trials [26, 27] may produce antibodies reactive to some or all commercially available EIA kits and may render supplemental Western blot testing useless, because these tests will also be positive. The use of plasma HIV RNA (virus load) assays instead of serologic EIA/ Western blot algorithms also may be problematic, because of the diminishing specificity of these assays when used as a screening tool [28–30]. Therefore, the development of testing strategies will be necessary to minimize the possibility for social harm for future HIV vaccine trial participants, to diagnose HIV infection in these trial participants without unblinding, and to facilitate reporting and treatment of those HIV infected in the presence of HIV vaccination.

Options for testing strategies include modifying HIV testing algorithms, incorporating a serologic marker into vaccine constructs, and creating new screening tests. Testing algorithms could be modified at the screening or testing level. At the screening level, vaccine recipients could be requested to self-identify and then be directed to a separate screening track with more extensive laboratory testing. At the testing level, persons who identify as vaccine recipients and have weak-reactive EIA and Western blot results would be asked to return within a short preset time period, and, if results remain unchanged or become nonreactive, these persons would be determined uninfected. A second solution requires the inclusion of a serologic marker into a vaccine construct that would easily identify a person as a HIV vaccine recipient on Western blot and indicate the need for further testing. Unfortunately, these strategies rely on or lead to the disclosure of vaccine trial participation that, for a phase III trial participant, could reveal other high-risk activities and potentially result in discrimination. Endorsing the development of new laboratory tests, such as an EIA that relies on less immunogenic epitopes that would be omitted from future HIV vaccine constructs, seems obvious and would avoid social harms, but, given the increasingly expanding arena of new constructs, this strategy would require
the continual development and evaluation of new EIA test kits and the access to multiple kits at HIV testing sites to cover various constructs.

Perhaps the most promising solution to date involves nucleic acid testing (NAT), a process that involves amplification of HIV-1 nucleic acid sequences, rather than relying on the presence of antibodies [31] as a screening test. In the past, NAT technology was used primarily in research or clinical diagnostic settings, but the development of commercially available qualitative assays may allow for more widespread use. The US FDA recently licensed the NAT system of the National Genetics Institute for screening of plasma donors and the NAT system of Gen-Probe (San Diego) for whole blood donations [32]. These systems and others may be of use during large-scale efficacy trials. However at present, demanding requirements, such as molecular technique training among laboratory staff, increased laboratory equipment and space allocation, a 12-h assay performance time, and cost [33] may prohibit their use in smaller diagnostic laboratories and resource-poor, international settings. Improvements in NAT or the development of other tests or testing algorithms will be essential in the future to correctly identify HIV-infected individuals and to continue accurate HIV reporting in the presence of HIV vaccine trials and vaccination programs.

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References


