

Performance of a Redesigned HIV Selectest Enzyme-Linked Immunosorbent Assay Optimized To Minimize Vaccine-Induced Seropositivity in HIV Vaccine Trial Participants

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Vaccine-induced seropositivity (VISP) or seroreactivity (VISR), defined as the reaction of antibodies elicited by HIV vaccines with antigens used in HIV diagnostic immunoassays, can result in reactive assay results for vaccinated but uninfected individuals, with subsequent misclassification of their infection status. The eventual licensure of a vaccine will magnify this issue and calls for the development of mitigating solutions in advance. An immunoassay that discriminates between antibodies elicited by vaccine antigens and those elicited by infection has been developed to address this laboratory testing need. The HIV Selectest is based on consensus and clade-specific HIV peptides that are omitted in many HIV vaccine constructs. The assay was redesigned to enhance performance across worldwide clades and to simplify routine use via a standard kit format. The redesigned assay was evaluated with sera from vaccine trial participants, HIV-infected and uninfected individuals, and healthy controls. The HIV Selectest exhibited specificities of 99.5% with sera from uninfected recipients of 6 different HIV vaccines and 100% with sera from normal donors, while detecting HIV-1 infections, including intercurrent infections, with 95 to 100% sensitivity depending on the clade, with the highest sensitivities for clades A and C. HIV Selectest sensitivity decreased in very early seroconversion specimens, which possibly explains the slightly lower sensitivity observed for asymptomatic blood donors than for clinical HIV cases. Thus, the HIV Selectest provides a new laboratory tool for use in vaccine settings to distinguish the immune response to HIV vaccine antigens from that due to true infection.

The ongoing development and testing of candidate HIV vaccines have resulted in a growing population of active and former trial participants with circulating antibodies to the vaccine antigens, which is an anticipated outcome of vaccine design in many cases. Induction of a strong humoral immune response is frequently a correlate of vaccine efficacy, and recent studies demonstrating the importance of antibody responses to HIV envelope antigens in the induction of protective responses mean that newer products will likely focus on increasing Env-specific antibody levels (1, 2). However, the detection of HIV vaccine-induced antibodies in standard serological diagnostic tests, designated vaccine-induced seropositivity (VISP) or seroreactivity, can result in misclassification of an individual's true HIV status if diagnostic tests are not conducted in a specialized laboratory using an algorithm that distinguishes VISP from true infection. This phenomenon has led to a variety of problems ranging from scientific to social, which have been documented in other reports (3–8) and were the focus of a recent meeting sponsored by the Global HIV Vaccine Enterprise and the National Institutes of Health (http://www.vaccineenterprise.org/sites/default/files/VISP%20Meeting%20Report_FINAL_0.pdf).

The frequency of VISP varies substantially as a function of the type of vaccine and the specific diagnostic test. Overall, 41.7% of HIV vaccine recipients in 27 HIV Vaccine Trials Network (HVTN)-sponsored HIV vaccine trials of 25 different vaccine products, conducted between 2000 and 2010, were found to ex-

hibit VISP at the trial exit date when VISP was surveyed across multiple serological kits (9). VISP frequencies ranging from 6% to as high as 80% to 100% have been associated with vaccination protocols employing adenovirus (9, 10) and modified vaccinia virus Ankara (MVA) (32), depending on which serological kits were used. In contrast, low levels of VISP were observed in RV144 vaccine recipients tested with the Vironostika HIV-1 kit, although the same kit yielded divergent results in other trials (8, 9) (HVTN, unpublished data). VISP peaked 6 months following the final vaccination, at which time seroreactivity was detected by the Vironostika kit in 28 (0.4%) of 7,015 tested vaccine recipients, compared to 4 (0.06%) of 7,099 tested placebo recipients (33). VISP frequency is also highly dependent on the specific assay system used, with frequencies ranging from 6% to 92% for the same group of serum samples tested with different kits in one extreme example (9). VISP can be a long-term problem; it has been shown to re-

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main for up to 15 years after vaccination, during which time the link to an individual's vaccination history may be lost (3, 9, 34).

VISP may have wide-reaching personal and societal consequences. In the United States, a positive HIV test result may lead to denial of employment, insurance coverage, or military service and precludes donation of blood, bone marrow, or other tissues (4, 5). The potential for other social harms has been raised in several prior studies (4–7, 11, 12). The perception of VISP as a long-term risk of participation also affects the recruitment of subjects into vaccine trials (<https://dlib.lib.washington.edu/dspace/handle/1773/23428>), with measurable attrition rates documented after counseling about such risks during participant screening (www.hvtm.org/meeting/ppt/may09/3/Mark.ppt). Following licensure and widespread use of an HIV vaccine, positive HIV test results due to VISP could skew HIV incidence data, making it more challenging and expensive to monitor the effectiveness of an HIV vaccination campaign and to correctly target antiretroviral therapy to individuals truly infected with HIV.

Current practice for diagnostic testing in HIV vaccine trials relies on multistep algorithms in which immunoassays typically are used for screening, followed by confirmatory viral load testing for initially HIV-reactive samples. While HIV Western blotting has traditionally been employed to confirm infections in nonvaccine settings, nucleic acid tests such as RNA viral load tests have been integrated into algorithms used in vaccine trials as confirmatory tests to resolve the status of initially reactive samples, including those with discordant or indeterminate results potentially due to VISP. The average cost of viral load assays has been reported to be over \$50 per test in resource-limited settings and higher in the United States (13–15). The availability of alternative methods that would simplify the testing algorithm and reduce the dependence on costly and technically demanding assays would simplify testing for recipients of experimental, and ultimately licensed, HIV vaccines.

To address the laboratory testing issues posed by VISP, the HIV Selectest, an enzyme-linked immunosorbent assay (ELISA), was developed based on the principle that the immune response to infection could be distinguished from that due to immunization using HIV peptide antigens not included in most HIV vaccine preparations (16, 17). These peptide antigens were initially selected from the cytoplasmic region of gp41 and the p6 gag region, which to date have not been reported to contribute to protective immunity but induce antibody responses in HIV infections. The peptide sequences also demonstrate relatively high conservation among group M HIV subtypes A to J.

The originally described Selectest has subsequently been modified to optimize performance within the parameters of a standardized kit format suitable for use in ongoing and future vaccine trials. The transfer of the Selectest technology into a commercial setting for kit development under good manufacturing practices (GMP) engendered various technical challenges whose resolution led to variations in assay performance with respect to the original ELISA. In this process, the peptide composition has been redesigned to improve sensitivity and specificity, while the overall procedure has been simplified in a reproducible robust format. The studies reported here describe the results of performance evaluations that challenged the ability of the redesigned Selectest to correctly distinguish VISP from HIV infection among uninfected HIV vaccine recipients, HIV-infected individuals, and control

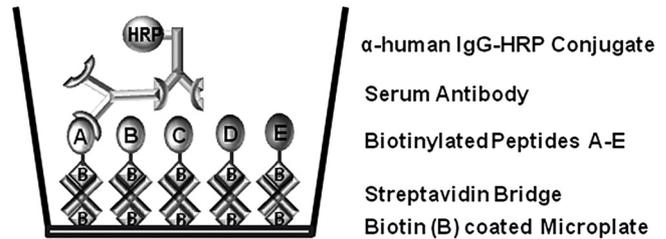


FIG 1 HIV Selectest assay configuration. A mixture of 5 biotinylated peptides is immobilized, via a streptavidin bridge, to the wells of a 96-well biotin-coated microplate. Serum antibodies are detected with a human IgG-specific horseradish peroxidase (HRP) conjugate and a chromogenic substrate.

subjects, including uninfected placebo recipients and blood donors.

MATERIALS AND METHODS

HIV Selectest. The redesigned HIV Selectest is an antibody-capture ELISA for human IgG antibodies employing a mixture of biotinylated peptide antigens immobilized in microplate wells via a streptavidin linkage (Fig. 1) (18). Detection of bound serum antibodies is mediated through an anti-IgG-horseradish peroxidase conjugate, whose binding is revealed colorimetrically with the soluble peroxidase substrate tetramethylbenzidine. HIV Selectest antigens included a mixture of consensus sequence peptides with applicability to multiple worldwide HIV clades plus a limited number of clade-specific peptide variants (19–22). The peptide sequences used in this Selectest assay (gp41-1, gp41-2, and Vpu) were limited to those not employed in most vaccine candidates evaluated in the past decade. Individual peptides were selected based on detection of a range of serum samples, including low-titer and low-affinity samples. A total of 5 peptides were selected as antigens, comprising gp41-1 and gp41-2 consensus peptides, a gp41-1 clade B variant, a gp41-1 clade C variant, and a Vpu peptide (Table 1). Of the peptides selected, Vpu was the most valuable contributor for clade C detection and gp41-1 clade B-fl was the most valuable contributor for clade B detection (data not shown). Human serum samples were tested at 1:100 dilution. A negative serum control was created by pooling sera from 7 normal healthy blood donors. The assay cutoff value was determined as the mean absorbance of four replicates of the negative serum control plus the fixed value 0.075, which was selected to be greater than 5 standard deviations from the mean and to yield specificity of >99%.

Assays were carried out using redesigned Selectest kits (Immunitics, Inc., Boston, MA), which included all assay materials, reagents, and positive- and negative-control sera. All steps were performed at room temperature (19°C to 25°C). A streptavidin-bovine serum albumin conjugate was added to the wells of a biotin-coated 96-well microplate, which was incubated for 60 min with agitation (the latter was subsequently shown to be unnecessary in this step and the next step). Following a brief rinse with 3 changes of wash buffer (phosphate-buffered saline with 0.05% Tween 20), biotinylated peptides were added and incubated for 30 min with agitation to immobilize them on the microplate. After a second similar buffer rinse, serum samples diluted 1:100 in sample diluent were added and incubated for 1 h without agitation. Microplates were then rinsed with 6 changes of wash buffer, and a 1:5,000 dilution of goat anti-human IgG-horseradish peroxidase conjugate was added to each well and incubated for 1 h without agitation. After an additional buffer rinse as described above, microplates were incubated with tetramethylbenzidine substrate solution for 8 min without agitation. An acidic stop solution was then added to each well, and absorbance was read on a microplate reader at 450 nm, with a reference wavelength of 650 nm. The absorbance index was calculated as the absorbance of the sample divided by the cutoff value. Absorbance index values of ≥ 1 were interpreted as positive, while absorbance index values of < 1 were interpreted as negative.

TABLE 1 HIV Selectest peptide antigen components

Peptide	Target clade	Peptide sequence
gp41-1	Consensus ^a	LIAARIVELLGHSSSLKGLRRGWEALKYLWNLLQYWQELKNSAISL
gp41-1 clade B-fl	B	LIAARIVELLG-----RRGWEALKYWNLLQYWSQELKNSAISL
gp41-1 clade C var 1	C	LIAARAVELLGRSSRLRGLQRGWEALKYLGSLVQYWGLELKKSAISL
gp41-2	Consensus	AVAEGTDRVIEVVQRVCRAILNI PRRI RQGFERALL
Vpu clade C	C	RAEDSGNESEGDTEELSTMVDMGHLRLLDNDL

^a Consensus sequences were designed to cover multiple clades.

Vaccine trials. Vaccine trials were selected on the basis of the availability of samples according to institutional review board approval and other constraints of the source institutions, to cover as wide a range of vectors, immunogens, and vaccine types as possible. VaxGen VAX003 and VAX004 trials were based on recombinant gp120 in B/E and B/B versions, respectively, administered with an alum adjuvant. These phase 3 trials enrolled 2,500 and 5,400 participants in Thailand and the United States, respectively (23, 24). HVTN039 (phase 1) tested a canarypox virus vector, ALVAC-HIV vCP1452, that encodes HIV-1 gp120, the entire Gag protein, a portion of Pol, and several human cytotoxic T lymphocyte (CTL) epitopes from Nef and Pol; HVTN203 (phase 2) also evaluated this vector in combination with recombinant gp120. Enrollments in HVTN039 and HVTN203 represented 110 and 330 participants, respectively, all within the United States (25, 26). HVTN065 employed a vaccine developed by GeoVax, containing pGA2/JS7 DNA vaccine as a prime combined with MVA/HIV65 as a boost. Encoded components were Gag, PR, RT, Env, Tat, Rev, Vpu, and Pol. This phase 1 trial enrolled 120 participants, all in the United States (27). HVTN204 (phase 2) studied a multiclade DNA prime-recombinant adenovirus 5 (Ad5) boost vaccine including Gag, Pol, Nef, and Env sequences. A total of 480 participants were enrolled in HVTN204, from which 251 samples from the United States and South Africa were tested in this study (28). HIVIS03 was a phase I trial investigating a DNA prime and MVA-CMDR boost regimen in which the formulation included multiclade sequences of gp160 and truncated Env, Gag, Pol, Rev, and RT, evaluated with 60 participants in Tanzania (29). RV144 used the poxvirus vector ALVAC-HIV vCP1521, containing *env*, *gag*, and *pol* genes, as a prime together with the AIDS VAX B/E recombinant gp120 protein as a boost. RV144 enrolled 16,402 participants in Thailand, representing the largest phase 3 HIV vaccine trial conducted to date (30).

Serum panels. Serum panels evaluated with the HIV Selectest comprised well-characterized HIV-positive serum samples representing all major HIV clades (671 samples), sera from HIV-infected asymptomatic blood donors (497 samples), sera from uninfected blood donor controls (400 samples), and sera from vaccine and placebo recipients (1,183 samples) in the HVTN065, HVTN039, HVTN203, HVTN204, HIVIS03, RV144, VAX003, and VAX004 trials, collected at the time of peak immune response (683 samples). HIV Western blot-confirmed serum samples of clinical origin (presumed clade B, United States) and well-characterized samples from HIV-infected patients were obtained from New York Biologics (Southampton, NY) and Bioreclamation Inc. (Westbury, NY), respectively. Samples from HIV-infected asymptomatic blood donors (presumed clade B) were received from a repository developed under the Retrovirus Epidemiology Donor Study program at the National Heart, Lung, and Blood Institute and made available via BioLINCC (<https://biolincc.nhlbi.nih.gov/home>), and from the American Red Cross (Rockville, MD), and ProMedDx Inc. (Norton, MA). HIV global panels were obtained from SeraCare (Milford, MA) and ZeptoMetrix (Buffalo, NY). HIV seroconversion panels were obtained from SeraCare. Serum panels were provided under material transfer agreements as follows: RV144, Jerome Kim and Charla Andrews (WRAIR); VAX003/VAX004, Faruk Sinangil and Carter Lee (Global Solutions for Infectious Diseases); Rwanda (clade A) and Zambia (clade C), Eric Hunter (Emory University/International AIDS Vaccine Initiative); South Africa (clade C), Barton Haynes (Duke University/Center for HIV-AIDS Vaccine Immunology)

and Sheila Keating (Blood Systems Research Institute); HIVIS03, Said About (Muhimbili University of Health and Allied Sciences). Samples from normal healthy non-HIV-infected blood donors were obtained from Creative Testing Solutions (Tempe, AZ). All samples from research studies that were tested in the present study were acquired under institutional review board approval from the source institutions.

Peptides. Biotinylated peptides were synthesized either by AnaSpec, EGT Group (Fremont, CA), or by JPT Peptide Technologies, GmbH (Berlin, Germany). Peptide sequences were verified by mass spectrometry, and purity of at least 90% was established by chromatographic analysis.

Statistical methods. *P* values were calculated with Fisher's exact test as two-sided values, unless otherwise indicated. The threshold for significance in comparisons of sensitivity and specificity values was 0.05. Confidence intervals (CIs) were calculated by the Clopper-Pearson exact method.

RESULTS

The sensitivity of detection of HIV-positive sera with the modified HIV Selectest assay was evaluated with panels including over 650 serum samples from HIV-infected blood donors and clinical patients in Rwanda, the United States, and South Africa, where the dominant clades are A, B, and C, respectively. The sensitivities of detection varied from 95% for U.S. samples (clade B) to 100% for Rwandan samples (clade A), with an average of 97.9% across all clades. The difference in the sensitivities of detection between clade B and the other clades was statistically significant ($P < 0.05$), while the sensitivities for clades A and C and those represented in the global panels were not significantly different ($P = 0.56$) (Table 2). Within the group of HIV-positive serum samples from U.S. sources, the observed sensitivities were 92% for sera from asymptomatic HIV-infected blood donors and 95% for HIV clinical case sera, a reproducible but not statistically significant difference ($P = 0.20$) (Table 3).

Serum samples from both vaccine and placebo recipients in 8 HIV vaccine trials, including HIVIS03, RV144, VAX003, VAX004, HVTN065, HVTN039, HVTN203, and HVTN204, were tested with the modified HIV Selectest. Rates of reactivity (VISP) based on the most reactive licensed HIV assays, as reported by the sponsors, ranged from 55% to 100% in these trials. Analysis of serum reactivity by HIV-1 Western blotting showed that, for these trials, VISP is typically due to antibody responses to p24 and gp160 (data not shown). In the HIV-uninfected vaccine recipient group, positive Selectest results were obtained for 1 sample in RV144, 1 sample in HVTN065, and 2 samples in HVTN039/HVTN203, a frequency of 0.6% among the 683 samples tested (Table 4). One of 150 preimmune samples and 2 of 255 samples from placebo recipients were also found to be positive by Selectest, yielding an overall frequency of 0.6% among 1,183 uninfected vaccine trial participants. The specificity in a panel of samples from 400 normal healthy donors was 100% (95% CI, 99.1% to 100%). The frequencies of positive results for vaccine and placebo recipients were

TABLE 2 Sensitivity of HIV Selectest for HIV-positive sera by clade

Presumed clade of infection	No. infected	No. detected positive	Sensitivity (% [95% CI]) ^a
Clade A (Rwanda)	100	100	100 (97.1–100)
Clade B (United States)	222	211	95.0 (91.3–97.5)
Clade C (South Africa, Malawi, and Zambia)	267	265	99.3 (97.3–99.9)
Global panels (clades A, B, C, D, E, F, G, J, and O and untyped)	81	80	98.8 (93.3–100)
Total in HIV-positive panels	671	656	97.9 (96.3–98.7)

^a Sensitivity was calculated as no. of serum samples positive in HIV Selectest/no. of infected individuals.

statistically identical ($P = 0.67$) and in sum were not distinguishable from that observed for normal healthy donors ($P = 0.24$). The low rate of positive assay results among uninfected vaccinated subjects confirmed the absence of vaccine-induced seropositivity as measured with the modified Selectest assay, in contrast to licensed HIV antibody tests. The discrimination of patients with HIV-1 infections from uninfected vaccine recipients and blood donors is illustrated by the distribution of absorbance values for these groups with the modified Selectest (Fig. 2).

Intercurrent infections primarily among participants in VAX003/VAX004 trials were detected with 97% sensitivity overall, varying insignificantly between vaccine and placebo groups ($P = 0.6$, one-sided) (Table 5). The sensitivity of the modified Selectest versus the recency of infection was investigated using a clade C serum panel (Blood Systems Research Institute) enriched in early infections, which were characterized with respect to recency by the Vitros LS assay, in which the signal/cutoff ratio is proportional to the titer of host antibodies (31). The modified Selectest sensitivity was 63% among 24 serum samples representing the very earliest infections (signal/cutoff ratios of <4), but the sensitivity increased to 96.4% (95% CI, 81.7% to 99.9%) overall in the remaining population of early and established infections, a significant difference ($P < 0.01$, one-sided) (Fig. 3). The modified Selectest similarly proved less sensitive than a licensed assay in detection of early seroconversion samples from commercial seroconversion panels, detecting only the terminal samples in 2 of 7 seroconversion panels tested. Samples from these panels that were reactive with gp160 on HIV-1 Western blots did not consistently react in the modified Selectest, suggesting that the envelope antigen does not invariably elicit antibodies to the C terminus of gp41 during the early seroconversion period.

DISCUSSION

The GMP modifications introduced into the HIV Selectest subsequent to its initial design resulted in several improvements, including an increase in sensitivity for sera from HIV-infected individuals, a decrease in nonspecific reactivity with sera from vaccine recipients, and simplification of the original assay, which had been carried out in two separate ELISA plates, into a single test plate. The increase in sensitivity was accomplished by supplementing the original consensus peptide sequences with new clade-specific peptide sequences that were selected based on frequency, as derived from the Los Alamos National Laboratory (LANL) database (<http://www.hiv.lanl.gov>), along with introduction of a clade C Vpu peptide. These changes resulted in improved sensitivity for

clade C in particular. Nonspecific reactivity was significantly reduced by elimination of the p6 peptide, which was one of the original antigen components but which was responsible for serological reactivity among uninfected vaccine recipients and controls (16) (E. Cormier, unpublished data, and O. Penezina, unpublished data). Proprietary immunochemistry allowed the combination of all peptide antigens into a single-well ELISA with a single cutoff value. The multi-peptide format is amenable to scaled-up production, for which the characteristics and reproducibility of the peptide mixture can be readily verified by probing the microplate with sera specific to each peptide as a quality control measure.

The modified HIV-Selectest demonstrated sensitivity in the 99 to 100% range for clade A and clade C HIV sera in chronic infection but slightly lower values for clade B. Given the limitations in the numbers and types of samples tested, the significance of these differences is not yet clear. Serum samples representing clades D to J, type O, HIV-2, and circulating recombinant forms were not available in statistically significant numbers in each category for this study and remain to be evaluated. Evaluations of seroconversion panels and HIV-positive serum samples of known recency show, however, that the sensitivity of the redesigned HIV Selectest decreases at the very earliest time points postinfection. This decrease may also explain the slightly lower sensitivity of detection of HIV-positive sera among asymptomatic infected blood donors versus HIV-infected clinical cases, assuming blood donors represent more-recent infections. While window-phase or recent infections are challenging to detect with any assay, the redesigned HIV Selectest, as a third-generation assay, appears slightly less sensitive than currently licensed fourth-generation HIV assays that incorporate antigen detection and additional immunodominant antigens. Nevertheless, the modified Selectest successfully detected the vast majority of intercurrent infections in the VaxGen VAX003/VAX004 trials, suggesting its suitability in practice for distinguishing true infections from VISP in a vaccine trial setting.

In this study, the capability of the HIV Selectest to distinguish VISP from true HIV infections was evaluated with serum samples from participants in trials of a diverse group of HIV vaccines, as well as HIV-infected individuals and controls. The vaccine trial group included vaccines both with and without sequence overlap between vaccine antigens and Selectest antigens derived from the gp41 region of the envelope protein; the nonenvelope Vpu peptide used in the Selectest is not included in the majority of candidate vaccines, to our knowledge, with the exception of the GeoVax vaccine.

Vaccines used in the VAX003/VAX004, HVTN039/HVTN203, HVTN204, and RV144 trials incorporated a truncated envelope protein, either gp120 or gp140, excluding the C-terminal region of gp41 where the Selectest antigen sequences are located. Otherwise,

TABLE 3 Sensitivity of HIV Selectest for HIV-positive clade B sera from HIV-infected asymptomatic blood donors versus HIV-infected clinical cases

HIV-infected sample source	No. infected	No. detected positive	Sensitivity (% [95% CI]) ^a
Blood donors	497	458	92.2 (89.4–94.4)
Clinical cases	222	211	95.0 (91.3–97.5)

^a Sensitivity was calculated as no. of serum samples positive in HIV Selectest/no. of infected individuals.

TABLE 4 HIV Selectest performance in uninfected vaccine trial participants and normal donors

Vaccine trial	VISP (%) ^a	No. Selectest positive/no. of samples ^b					Specificity for all samples (% [95% CI])
		Preimmune	Placebo, peak response	Vaccine recipients, peak response	Vaccine recipients, nonpeak	Total	
HVTN065	29–76	NA	1/17	1/53	NA	2/70	97.1 (90.1–99.7)
HIVIS03	100	NA	NA	0/29	NA	0/29	100 (90.2–100)
RV144	NA	1/150	0/40	1/170	NA	2/360	99.4 (98.0–99.9)
HVTN204, South African arm	3–85	NA	0/33	0/34	NA	0/67	100 (95.6–100)
HVTN204, U.S. arm	8–87	NA	0/92	0/92	NA	0/184	100 (98.4–100)
VAX003/VAX004	NA	NA	NA	0/86	0/95	0/181	100 (98.4–100)
HVTN039/HVTN203	0–55	NA	1/73	2/219	NA	3/291	99.0 (97.0–99.8)
Total in vaccine trials	NA	1/150	2/255	4/683	0/95	7/1,183	99.4 (98.8–99.8)
HIV-negative normal donors	NA	0/400	NA	NA	NA	0/400	100 (99.1–100)

^a Data provided by HVTN represent the range of VISP rates observed with different HIV test kits. NA, not available.

^b Positive samples were retested in duplicate and were confirmed positive based on two of three results.

the vaccine regimens were disparate; the VaxGen VAX003/VAX004 trials made use of alum as an adjuvant to a recombinant protein, while HVTN204 evaluated a DNA prime and adenovirus 5 (Ad5) vector boost, and HVTN039/HVTN203 and RV144 both used canarypox as a vector with recombinant gp120 envelope protein. A common element, however, was the observation of significant end-of-study frequencies of VISP associated with both adenovirus- and pox-based vaccines (9); these ranged from 0% to 55% for HVTN203 and from 3% to 87% for HVTN204, depending on the test (HVTN, unpublished data). In contrast, testing of vaccine and placebo recipients from the six trial regimens with the modified HIV-Selectest yielded an overall frequency of reactivity of <0.5%. Thus, as predicted, the modified HIV-Selectest shows negligible reactivity with sera from recipients of vaccine formulations lacking antigenic components common to both vaccines and the Selectest.

Two vaccine trials, i.e., HIVIS03 and HVTN065, employed prime-boost strategies involving use of a full-length envelope-based DNA vaccine to prime, followed by an MVA vector boost. In both cases, the DNA sequence of the prime extended through the

C-terminal region containing the Selectest gp41 peptides, implying direct homology between vaccine and assay antigen sequences. The gp120/140 envelope sequence used in the MVA boost, however, was truncated before the C terminus of gp41 and thus did not overlap the Selectest peptides. Both vaccine regimens elicited high levels of VISP in licensed HIV screening tests performed at the end of the study, i.e., 29% to 76% in HVTN065 (HVTN, unpublished data) and 100% in HIVIS03 (32), depending on the test used. In the latter trial, VISP developed only after the MVA boost, suggesting that the envelope protein was significantly more immunogenic in that presentation than as produced via the DNA vaccine. This is consistent with the generally observed low immunogenicity of DNA vaccines. Within these limitations, however, the present study demonstrates that HIV DNA vaccines containing sequences directly homologous to the HIV-Selectest envelope peptides do not appear to generate antibodies to these peptides that are detected by the Selectest. This finding supports a potential role for the Selectest in future trials of such vaccines that are planned or under way, as well as others that similarly may involve full-length envelope DNA constructs.

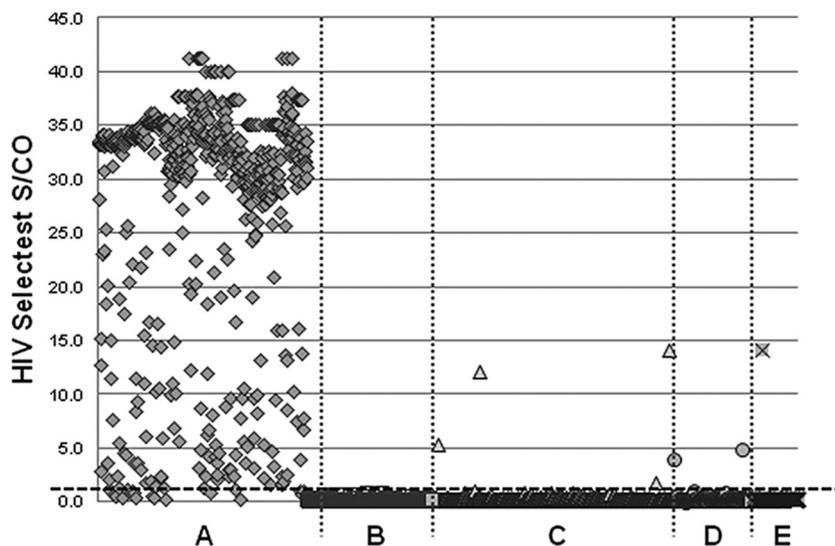


FIG 2 HIV Selectest signal/cutoff (S/CO) values for 671 HIV-1-infected individuals (A, \diamond), 400 normal blood donors (B, \square) and 1,183 uninfected HIV vaccine trial participants, including 778 vaccine recipients (C, \triangle), 255 placebo recipients (D, \circ), and 150 preimmune subjects (E, \boxtimes). The cutoff value is indicated as a horizontal dashed line.

TABLE 5 Detection of intercurrent HIV infection by HIV Selectest among vaccine trial participants

Vaccine trial	Vaccination status	<i>n</i>	HIV status	No. Selectest positive	% Selectest positive (95% CI)	Average % positive (95% CI)
VAX003/VAX004	Placebo	105	Infected	101	96.2	97.0
	Vaccine	160	Infected	156	97.5	
HVTN204, South African arm	Placebo	NA	NA	NA	NA	100
	Vaccine	1	Infected	1	100	
HVTN203/HVTN039	Placebo	1	Infected	1	100	100
	Vaccine	1	Infected	1	100	
Total	Placebo	106	Infected	102	96.2 (90.6–99.0)	97.0 (94.2–98.7)
	Vaccine	162	Infected	158	97.5 (93.8–99.0)	

Overall, our data demonstrate that the HIV-Selectest detects antibodies in sera from HIV-infected individuals but not HIV vaccine recipients or controls, suggesting that this assay may be a useful adjunct to laboratory diagnostic algorithms in HIV vaccine trials or poststudy testing, either to eliminate detection of VISP at initial serological testing or to distinguish it from true infection in a subsequent confirmatory step. The eventual licensure of an HIV vaccine is likely to underscore the call for such a test to be implemented in the broader community. The potential impact of vaccination on HIV testing is illustrated by examining the predictive value of current tests in this scenario. In a model in which 5% of the population is vaccinated and HIV prevalence is 12% (predominantly clade C), as may be envisioned for regions with high levels of endemicity in southern Africa for instance, the positive predictive value of licensed HIV assays would average 85%, based on published data (9), versus 96% for the HIV-Selectest; with a vaccination rate of 20%, the positive predictive value decreases to 59% for licensed assays while remaining at 96% for the HIV-Selectest. Based on this projection and the findings reported here, further evaluations of the cost-effectiveness of algorithms incorporating the HIV-Selectest in comparison with current practices

for monitoring of vaccine trial participants and evaluation of outcomes are warranted.

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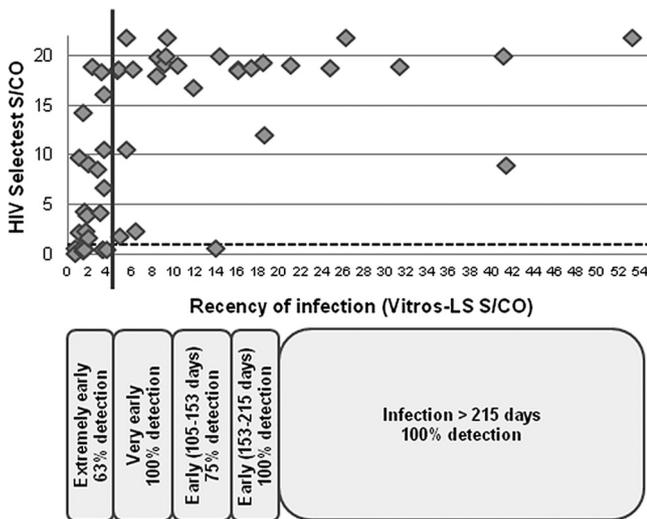


FIG 3 HIV Selectest signal/cutoff (S/CO) values versus infection recency in the clade C early-infection panel. The extremely early group (signal/cutoff values of <4) is to the left of the vertical line. The cutoff value is indicated by the horizontal dashed line.

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