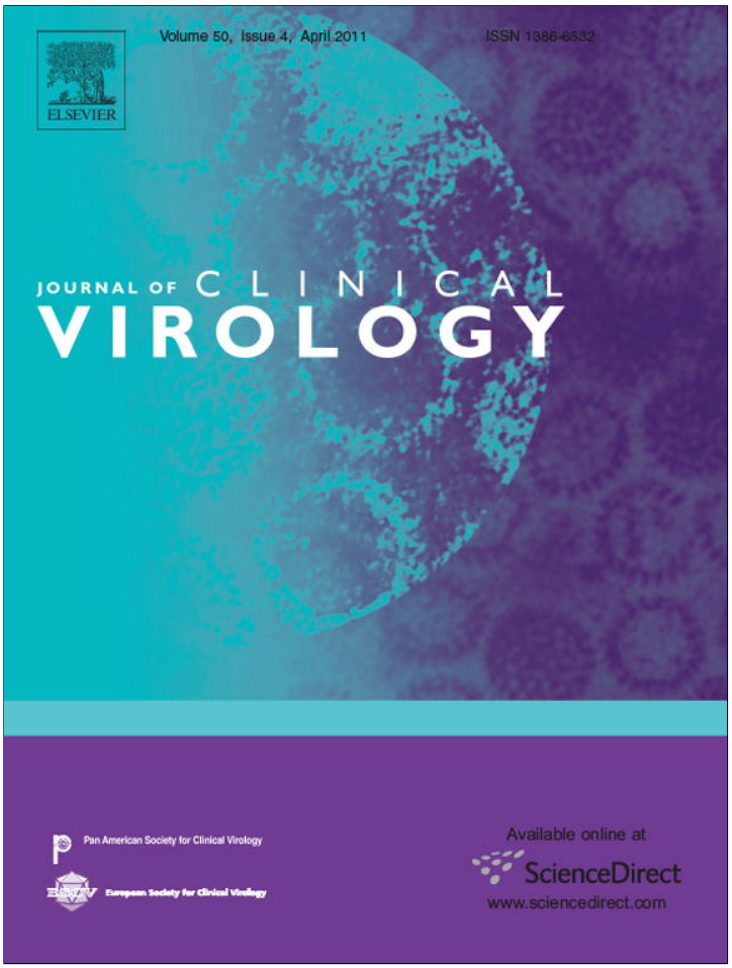

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Short communication

Vaccine-induced HIV seropositivity: A problem on the rise

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ABSTRACT

Background: Vaccine-induced antibodies to envelope proteins frequently cause HIV seroconversion in uninfected recipients of HIV vaccine candidates and may thus have an impact on the vaccinee's ability to donate blood or acquire a life insurance policy.
Objective: To determine the occurrence of positive test results when commonly used HIV immunoassays are used to screen sera of HIV-uninfected volunteers who received an adjuvanted HIV-1 vaccine candidate containing HIV-1 antigens p24, reverse transcriptase, Nef and p17.
Study design: Sera of 50 subjects who received this polyprotein vaccine in a single center in Belgium were tested with 6 HIV screening assays and 1 confirmation test. All samples were drawn one year after the administration of the first of two vaccine doses given with one month interval.
Results: Forty-five (90%) sera showed a positive test result in at least one of the 7 HIV tests used. The positivity rates were 88% in the Elecsys HIV Combi assay, 74% in the ADVIA Centaur EHIV and 48% in the PRISM HIV O Plus assay.
Conclusions: Interpretation of HIV test results is becoming increasingly complex with the growing number of volunteers participating in prophylactic HIV vaccine trials worldwide and the rising number of viral antigens included in these vaccine candidates. The results of this study in recipients of a highly immunogenic adjuvanted polyprotein HIV vaccine candidate devoid of envelope proteins, illustrate the increasing need for approaches that can discriminate HIV infection-induced antibodies from those elicited by a vaccine.

1. Background

Since 1987, more than 40,000 volunteers received human immunodeficiency virus (HIV) vaccine candidates worldwide.[1] Vaccine-induced seropositivity (VISP) is a common outcome of HIV vaccine trials,[2] mostly associated with vaccines containing envelope inserts.[2–6]

Current HIV screening policy relies on highly sensitive enzyme immunoassays detecting antibodies against different proteins, most often p24, HIV-1 gp41 and HIV-2 gp36. Although 4th generation tests reduce the size of the diagnostic window by combining HIV antibody and p24 antigen detection,[7–10] many clinical laboratories still use 3rd generation assays. Western blot or line immunoassay (LIA) confirmation tests are also antibody-based,[11,12] and may therefore also turn positive after receipt of multi-antigenic HIV vaccine candidates.[3]

Since the focus of HIV vaccine research has broadened from neutralizing antibody-inducing approaches to T-cell-inducing vaccines, increasing numbers of volunteers are receiving vaccine candidates containing compound HIV protein inserts. We were recently confronted with positive HIV screening test results in two uninfected recipients of a highly immunogenic HIV-1 vaccine candidate, consisting of the fusion protein F4 (p24-reverse transcriptase (RT)-Nef-p17) and the AS01$_B$ Adjuvant System.[13] All study participants were at low risk of acquiring HIV infection, screened twice with the 4th generation AxSYM HIV Ag/Ab Combo test before immunization, and remained seronegative after receipt of the study vaccine.

2. Objective

To determine the occurrence of VISP in commonly used HIV immunoassays in HIV-uninfected volunteers who received an adjuvanted HIV-1 vaccine candidate containing p24, RT, Nef and p17.

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Table 1
Antibody and antigen detection profile of the HIV immunoassays.

Immunoassay	Manufacturer	Detection profile[a]				
		HIV-1 Ab			HIV-2 Ab	HIV Ag
		Env	Gag	Pol	Env	
4th generation screening tests						
AxSYM HIV Ag/Ab Combo	Abbott	gp41			gp36	p24
Murex HIV Ag/Ab Combination	Abbott	Env[b]		Pol[c]	Env[b]	p24
Elecsys HIV Combi	Roche	gp41		RT	gp36	p24
3rd generation screening tests						
AxSYM HIV 1/2 gO	Abbott	Env[b]	p24		Env[b]	
PRISM HIV O Plus	Abbott	Env[b]	p24		Env[b]	
ADVIA Centaur EHIV 1/O/2	Siemens	gp41	p24		gp36	
Confirmation test						
INNO-LIA HIV I/II Score	Innogenetics	gp41, gp120	p17, p24	p31	gp36, gp105	

[a] HIV antibodies (Ab) or antigens (Ag) detected by the immunoassays.
[b] Envelope antigen detected by the assay, not further specified.
[c] Polymerase antigen detected by the assay, not further specified.

3. Study design

Samples. Serum samples from the PRO HIV-005 trial (NCT00434512), conducted at Ghent University and Hospital (Belgium), were examined.[13] 180 healthy HIV-uninfected volunteers received one month apart two doses of an HIV-1 vaccine candidate from GlaxoSmithKline Biologicals (Rixensart, Belgium), containing 10, 30 or 90 μg of F4 (p24-RT-Nef-p17) recombinant protein, adjuvanted with $AS01_B$ or reconstituted with water for injection (WFI). Fifty sera, drawn one year after administration of the first dose, were selected from the different dose groups based on F4 antibody concentrations, to ensure a balanced representation of low, medium and high antibody levels.

Assays. IgG antibody responses to F4, p24, p17 and RT were assessed by validated in-house enzyme-linked immunosorbent assays (ELISA).[13] The 4th generation AxSYM HIV Ag/Ab Combo test (Abbott Diagnostics, Germany) was used as a screening test during the clinical trial.[14] Serum samples were re-tested with five additional HIV screening assays, selected based on their common use and detection properties (Table 1).[15] The test panel included two 4th generation assays, Murex HIV Ag/Ab Combination (Abbott)[16] and Elecsys HIV Combi (Roche Diagnostics, Germany),[17] and three 3rd generation assays, AxSYM HIV 1/2 gO, PRISM HIV O Plus (Abbott)[14,18] and ADVIA Centaur HIV 1/O/2 Enhanced (EHIV) (Siemens Medical Solutions Diagnostics, Germany).[19] For confirmatory testing, all 50 samples were analyzed with the INNO-LIA HIV I/II Score assay (Innogenetics, Belgium) (Table 1). Trained laboratory personnel scored the reactivity pattern for each individual antigen on a scale from "−" to "4+", according to the manufacturer's instructions.[12,20]

Statistical analysis. For tests detecting antibodies to corresponding antigens, the association between in-house ELISA antibody concentration and commercial test results was measured using Spearman correlation in PASW Statistics 18.

4. Results

Table 2 provides an overview of all test results. All 50 samples scored negative in the AxSYM and Murex assays. In the PRISM assay, 25 subjects (50%) tested negative and 24 (48%) scored positive. In the ADVIA test, 37 samples (74%) showed reactivity. Significant correlations ($p < 0.001$) were observed between p24 antibody concentrations in the in-house ELISA and each of two commercial kits able to detect p24 antibodies (PRISM and ADVIA, $\rho_S = 0.76$ and 0.74, respectively). In the Elecsys test 44 subjects (88%) were considered seropositive based on the presence of anti-RT antibodies. A strong correlation ($p < 0.001$) was observed between the Elecsys results and the RT antibody concentrations in the in-house ELISA ($\rho_S = 0.77$).

None of the 50 samples fulfilled the criteria for a positive confirmation test, but 41 (82%) were scored as "indeterminate" (Table 2). Antigen p17 induced the weakest antibody responses, in line with the in-house p17 ELISA ($p = 0.004$, $\rho_S = 0.40$). For p17, 41 samples were scored negative, 5 "±" and 4 "1+". Higher responses were observed against p24, with only 8 negatives, 1 "±", 5 "1+", 16 "2+" and 20 "3+". The p24 LIA scoring correlated significantly ($p < 0.001$) with the in-house ELISA p24, the PRISM and ADVIA results ($\rho_S = 0.83$, 0.69 and 0.74, respectively).

5. Discussion

One year after receipt of an immunogenic HIV-1 vaccine containing no envelope proteins, 90% of the HIV-uninfected vaccinees scored positive in at least 1 out of 6 commonly used HIV screening assays. The seronegative subjects originated from the non-adjuvanted control groups in which the lowest antibody titers were measured. The frequency of positive test results in this study largely exceeds that observed in a previous study examining Env-based vaccines in which only 20.4% of the selected vaccine recipients reacted on at least 1 of 6 HIV screening tests.[3] It also exceeds the VISP rate of 41.7% recently reported by the HIV Vaccine Trials Network[2] and of 41% induced by adenovirus type 5 vectored *gag(/pol/nef)* vaccines.[21] Our data therefore strengthen the concern that recipients of immunogenic and increasingly complex HIV vaccine candidates may be misclassified as HIV-infected with the current screening assays.

Because of the absence of anti-envelope antibodies, none of our participants displayed a positive HIV confirmation test. However, 82% showed indeterminate test results on INNO-LIA due to reactivity with p24 and, to a lesser extent, p17 antigen. This may trigger unnecessary concern and repeated testing, or elicit the use of more expensive techniques such as polymerase chain reaction (PCR) to exclude true HIV infection. This is illustrated by the experience of a study participant who recently underwent routine HIV testing in the United States. A positive HIV screening test result was confirmed by reactivity to p65, p55 and p51 in one Western blot and to gp40, p24 and p18 in another. The absence of reactivity towards gp41, gp120 and gp160, together with a negative HIV PCR result, suggests that gp40 reactivity was due to low test specificity. Future vaccine candidates containing envelope protein(s) in addition to F4 may induce anti-envelope antibodies, thereby further limiting the usefulness and discriminatory capacity of current confirmation tests.

Table 2
Results of the HIV screening and confirmatory tests in 50 serum specimens from uninfected HIV vaccine recipients.

Immunoassay	Positive result [*n* (%) of subjects]	Negative result [*n* (%) of subjects]	Indeterminate result [*n* (%) of subjects]
Single antibody detection tests			
In-house anti-F4 ELISA	44 (88)	6 (12)	0
In-house anti-p24 ELISA	36 (72)	14 (28)	0
In-house anti-p17 ELISA	7 (14)	42 (84)	1 (2)
In-house anti-RT ELISA	33 (66)	17 (34)	0
4th generation screening tests			
AxSYM HIV Ag/Ab Combo	0	50 (100)	0
Murex HIV Ag/Ab Combination	0	50 (100)	0
Elecsys HIV Combi	44 (88)	6 (12)	0
3rd generation screening tests			
AxSYM HIV 1/2 gO	0	50 (100)	0
PRISM HIV O Plus	24 (48)	25 (50)	1 (2)
ADVIA Centaur EHIV 1/O/2	37 (74)	13 (26)	0
Confirmation test			
INNO-LIA HIV I/II Score	0	9 (18)	41 (82)

HIV vaccine recipients may experience stigmatization or discrimination in situations where an HIV test is determining important decisions, such as donating blood, obtaining a life or health insurance, or for purposes of immigration or employment.[4,22,23] In Belgium, the PRISM HIV O Plus test is used for the centralized screening of blood donors, in combination with HIV nucleic acid testing (NAT) of pooled samples. A positive screening test result, as observed in 48% of subjects in this study, will automatically lead to exclusion from blood donation, irrespective of NAT and confirmation test results. To avoid such situations, all study participants received a letter explaining that future HIV testing can give false positive results, and a certificate of trial participation containing information for physicians on the content of the vaccine, together with contact details of the investigator and the sponsor.

This and preceding studies clearly demonstrate that HIV test manufacturers should provide complete information about composition and antibody detection profile of their products. Laboratories using these screening tools should be well informed about qualities and limitations thereof. Participants in prophylactic HIV vaccine trials should be selected with care and they as well as their health care providers need to be adequately informed about the vaccine content and consequences of participation. Finally, this study highlights the need for the development of new testing strategies,[24,25] allowing for a quick and reliable differentiation between vaccine-induced immunity and true HIV infection.

Conflict of interest declaration

Eva Van Braeckel and Frédéric Clement declare no potential conflict of interest. Geert Leroux-Roels was principal investigator of clinical studies of a variety of candidate vaccines for Baxter, GlaxoSmithKline Biologicals, Novartis and SanofiPasteur. The Ghent University and University Hospital received sponsorship for the conduct of these studies, and GlaxoSmithKline Biologicals was the study sponsor for this clinical trial. Geert Leroux-Roels has also performed consulting services for GlaxoSmithKline Biologicals and Novartis. Patricia Bourguignon, Marguerite Koutsoukos and Lisa McNally were all employees at GlaxoSmithKline Biologicals at the time of study. The PRO HIV-005 trial (NCT00434512) was approved by the local independent ethics committee (Ghent University Hospital, Belgium) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all subjects prior to study entry.

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