Human Immunodeficiency Virus (HIV) Antigen-Antibody Combination Assays: Evaluation of HIV Seroconversion Sensitivity and Subtype Detection

Recently, 4th generation assays that permit the combined detection of human immunodeficiency virus type 1 (HIV-1) p24 antigen (Ag) and anti-HIV antibody (Ab) were introduced on the international market. The diagnostic window is reduced on average by 4 days with this new assay generation in comparison to 3rd generation antibody-screening enzyme immunoassays (EIAs) in HIV-1 group M subtype B primary infections (1, 6). Up to now, no data had been available on the sensitivity of this new generation of assays for detection of HIV-1 non-B subtype primary infection.

Dilution series of virus lysates of HIV-1 group M non-B subtypes, group O, and HIV-2, in HIV-negative serum are used as a substitute for seroconversion panels for the assessment of the influence of genetic diversity on the sensitivity of 4th generation HIV assays. This seems a valuable approach, since the sensitivity of 4th generation assays in seroconversion panels correlates with sensitivity for Ag detection in virus lysates at least for HIV-1 subtype E primary infection (unpublished data). However, the results from dilutions of virus lysates must be interpreted with caution if a limited number of virus stocks are tested.

The sensitivities for HIV Ag detection of one prototype and six commercially available HIV Ag-Ab (4th generation) combination assays were evaluated by testing virus stocks ($n = 10$) of different HIV-1 group M subtypes, group O, and HIV-2 diluted in HIV-negative serum adjusted to concentrations ranging between 5 and 15 pg of p24 Ag per ml according to the Dupont standard (Table 1). The sensitivities of the 4th generation assays were highly variable. The best performance was obtained with the VIDAS HIV DUO Ultra and Murex HIV Ag-Ab combination, which detected 7 of 10 virus lysates. Four assays were not able to detect HIV Ag in any 1 of the 10 isolates. The failure of VIDAS DUO HIV

Ultra to detect HIV-1 subtype C Ag under a concentration of 34 pg of p24 Ag per ml (which corresponds appreciatively to 125 pg of HIV Ag per ml) as observed by Ly et al. (2) could not be confirmed by our investigation. In a previous study from our group (6), VIDAS HIV DUO Ultra also showed a high sensitivity for Ag detection in virus isolates of different subtypes (A to H and group O). Its sensitivity for two HIV-1 subtype C isolates was equivalent to that of a stand-alone Ag assay with a detection threshold of 3 pg of p24 Ag per ml. The results of dilutions of HIV-1 subtype virus lysates are dependent on the strain used and cannot be generalized for all isolates of an HIV-1 subtype. In this context, it should be underlined that VIDAS HIV DUO Ultra failed to detect one HIV-1 group O isolate at a concentration of 6 pg of p24 Ag per ml (Table 1). However, in a previous evaluation, the assay showed a more than fourfold-higher sensitivity than the comparative stand-alone Ag assay for two of three group O isolates. Conclusions on the sensitivity of Ag detection from different HIV-1 subtypes can only be drawn by testing larger numbers of virus stocks than only one isolate of each subtype.

Ly et al. tested the sensitivity of Ab detection to HIV variants by using dilution series of anti-HIV-1- and anti-HIV-2-positive serum samples. While dilution series of virus lysates are appropriate to challenge the sensitivity of the Ag detection module of 4th generation assays, the results obtained from dilutions of anti-HIV Ab-positive samples very probably do not reflect the sensitivity of the Ab detection module of these assays. The results correlate with the absolute number of HIV Ab molecules present in the serum; however, the method, which detects the lowest concentration of HIV Abs, may not necessarily show the highest sensitivity for seroconversion samples (4; B. J. Weiblen, J.

TABLE 1. End point titration of HIV-infected cell culture supernatants

Culture supernatant	Titer of p24 Ag in pg/ml (pg of HIV Ag/ml) ^a	Sub- or serotype	Test result, expressed as index (signal/cutoff value) ^b									
			VIDAS HIV DUO Ultra	Murex HIV Ag-Ab combination	Cobas Core HIV Combi	VIDAS HIV DUO	Genscreen Plus HIV Ag-Ab	Enzygnost Integral	Vironostika HIV Uniform II Ag-Ab	Cobas Core HIV Ag EIA	Elecsys HIV Ag	Coulter HIV-1 p24 Ag
MP33	8 (29)	A	2.28	0.96	1.14	0.76	0.34	0.12	0.55	2.74	2.09	2.48
MP77	5 (18)	B	1.12	0.60	0.61	0.60	0.41	0.10	0.53	1.28	1.34	1.59
MP37	6 (22)	C	1.96	1.16	0.52	0.48	0.31	0.05	0.54	1.50	1.65	1.49
VI824	11 (40)	D	3.04	1.20	1.41	0.80	0.39	0.17	0.49	3.25	2.76	2.92
MP48	5 (18)	E (CRF A/E)	1.24	0.52	0.67	0.56	0.32	0.10	0.55	1.39	1.47	0.58
VI1310	9 (33)	F	1.24	1.19	1.14	0.60	0.44	0.31	0.54	2.59	2.24	1.81
VI1197	8 (29)	G	1.72	1.20	0.95	0.60	0.28	0.28	0.56	2.19	1.93	0.66
VI991	6 (22)	H	0.2	1.98	1.23	0.32	0.34	0.13	0.50	2.97	1.53	1.84
MP331	6 (22)	O	0.68	1.16	0.88	0.40	0.29	0.10	0.52	2.12	1.65	0.47
VI390	15 (54)	HIV-2	0.36	1.65	0.44	0.24	0.15	0.06	0.47	1.80	3.06	0.12

^a Calibrated with Dupont standard material (1 pg of p24 Ag corresponds to 3.6 pg of total HIV Ag).

^b A value of ≥ 1 is considered positive.

Acker, P. E. Garrett, R. T. Schumacher, supplemental technical bulletin, Boston Biomedica, Inc., Boston, Mass., 1993). Assays that use large recombinant Ags with multiple Ab binding sites show a higher sensitivity than those using short synthetic peptides. For screening purposes, it is most important for assays to have the antigenic epitopes most strongly recognized earliest in seroconversion and persistently recognized throughout infection. Endpoint titration of anti-HIV-positive samples should not be used to evaluate test kit sensitivity.

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Authors' Reply

In the April issue of the *Journal of Clinical Microbiology*, Dr. Weber et al. published the paper "Evaluation of a new combined antigen and antibody human immunodeficiency virus screening assay, VIDAS DUO Ultra" (2). In this publication, Dr. Weber et al. presented data on "end point titration of HIV-infected culture supernatants" in Table 4 of the article. Apparently the same observations are presented again in his letter to the editor, the only difference being that instead of end point titers, p24 viral antigen concentrations for each subtype are calculated by using calibrated standards. In the discussion section of his article, Dr. Weber has addressed the difference between our evaluation of VIDAS DUO Ultra combination assay for its inability to detect HIV-1 subtype C viral antigen (1) and his observation in his letter demonstrating detection of HIV-1 subtype C antigen by the same assay. He claims the difference in observations is "dependent on the strain used and cannot be generalized for all isolates of an HIV-1 subtype."

In his letter, Dr. Weber points out that "In a previous

study from our group (6) [cited here as reference 2], VIDAS HIV DUO Ultra also showed a high sensitivity for Ag detection in virus isolates of different subtypes (A to H and group O)." Furthermore, Dr. Weber points out that the results of dilutions are dependent on the individual viral strain used, and he uses Table 1 to show that in this evaluation. He shows that the VIDAS HIV DUO Ultra assay lacks sensitivity against an HIV-1 group O isolate (0.68 S/CO) or subtype H (0.2 S/CO), in contrast to a previous evaluation in which HIV-1 group O p24 apparently was adequately detected. We can only agree with Dr. Weber that the VIDAS HIV DUO Ultra assay may indeed be differentially sensitive to p24 from diverse HIV strains. In this study, he demonstrated differential sensitivities to HIV-1 subtype H and an HIV-1 group O strain (compared to his previous publication), and in our study, we have demonstrated a differential sensitivity to a subtype C strain. In contrast, AxSYM Combo has demonstrated equivalent sensitivities to all group M subtype strains and group O strains tested. While we agree that it would be ideal to use a large number of subtype samples to define antigen sensitivity, an inability to detect even one strain (in this case, HIV-1 subtypes C and H, as well as group O) indicates the lack of overall sensitivity to detect some antigen-positive samples. In other words, some HIV-1 antigen-positive samples will not be detected by VIDAS DUO Ultra assay.

In our publication (1), we did not use the viral lysate dilution series as a substitute for seroconversion panels. We used the viral lysate in addition to seroconversion series. Of the panels shown in detail in Table 2 of our paper, all but one is an "antigen-first" or "antigen-only" panel. A dilution series is a perfectly legitimate means of assessing the end point antigen sensitivities of different assays. Furthermore, unless commercial seroconversion panels of HIV-1 subtypes A, C, D, F, and G, etc., or HIV-1 group O are available, there is no alternative other than to estimate antigen (core protein) sensitivity against lysed virus. The same situation occurs for assessment of antibody sensitivity for HIV-1 non-B subtypes, HIV-1 group O, and HIV-2. Commercially available antibody seroconversion panels are exclusively subtype B (to our knowledge), and the only means of assessing end point antibody sensitivities is by dilution series of antibody-positive HIV-1 non-B subtypes, HIV-1 group O, and HIV-2. It is understood that the preferable method is to test non-B seroconversion panels to assess seroconversion sensitivity.

In addition, regarding detection of antibody to HIV strains, in our publication (1), we demonstrated that two of the seven HIV antigen-antibody combination assays did not detect one HIV-1 subtype C undiluted antibody-positive sample. We agree with Dr. Weber that the antigenic epitopes most strongly recognized during early seroconversion are essential in all screening assays. However, our data in the previous publication (1), as well as additional unpublished observations, demonstrate that several assays that relied on use of short peptides for antibody detection not only could not detect some undiluted samples, but also did not detect diluted samples efficiently. During early seroconversion, there are several factors that need to be considered in terms of an immune response. These include the presence of a low antibody titer or low-affinity antibodies to immunodominant regions. Our observations support the view that since there is high sequence variation among HIV isolates, assays that use large recombinants (with additional epitopes, including those strongly recognized during early seroconversion) have better

sensitivity for detection of all antibody-positive samples than those that rely on a short peptide or peptides and a single epitope.

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