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Western Blot Index for estimating recency of HIV infection

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BACKGROUND

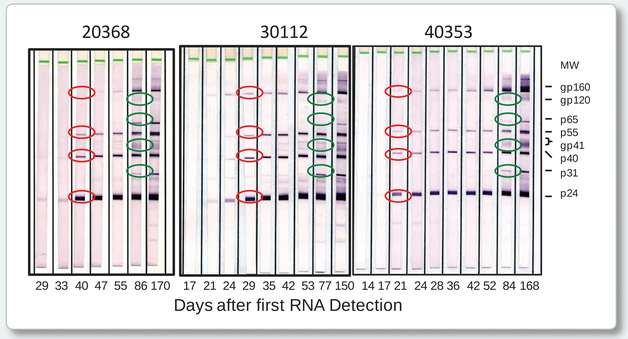
Estimation of time of HIV infection can be useful in epidemiological studies of incidence of infection, in establishing a link to probable risk behavior, and in narrowing the search for likely partners to whom infection could have been passed. Current HIV Recency assays such as the BED CEIA and HIV-1 Limiting Antigen (LAg)-Avidity EIA have limitations due to variable immune response among individuals, variability in different HIV-1 subtypes, and false recency rates in some individuals. Western Blot profiles show evolution of immune response to individual HIV antigens over time post infection. In this study, we examined sequential sampling from recently HIV infected individuals with known time of infection to determine whether progressive evolution of Western Blot (WB) reactivity can be utilized to estimate time of infection. The WB profiles of closely acquired samples from newly infected individuals were used to generate a WB Index (WBI) for a useful measure of recency of infection.

MATERIALS AND METHODS

Individuals at high risk of HIV infection in Kenya, Uganda, Tanzania and Thailand were monitored twice weekly by HIV RNA Aptima assay (Hologic, La Jolla, CA), the earliest available indicator of infection (the RV217 ECHO trial). Blood from first time RNA positive individuals was collected at frequent intervals after infection and tested by Western Blot (Bio-Rad, Hercules, CA). The intensity of each band was scored on a scale of 0, 0.5, 1.0, or 2.0 (absent, weak, medium and strong), and individual antigen intensities or sums of reactivity plotted as time after first detection of infection. This population represents HIV subtypes A1, A1C, A1D, A1CD, B, C, and CRF01_AE subtypes.

RESULTS

Figure 1.



Three seroconversion series showing the evolution of Western Blot antigen reactivity at various times points after first detection by RNA. Antibodies to p24, p40, p55, and gp160 (Early Bands – Red Circles) develop quickly after infection, with maximum reactivity by 21-35 days post infection. A more slowly evolving response is seen for p31, gp41, p68, gp120 (Late Bands – Green Circles) which appear by 75-85 days and continue to increase in intensity past 180 days post infection.

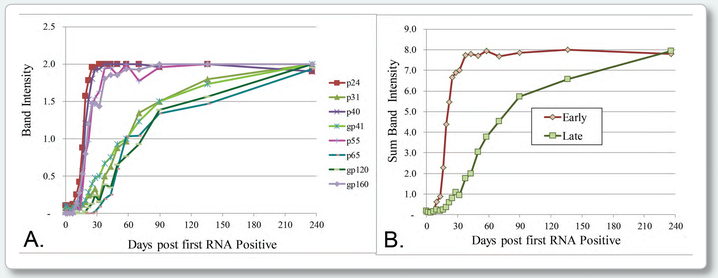
PIN	Day	p24	p31	p40	gp41	p55	p65	gp120	gp160	Early	Late
30112-B	0	0	0	0	0	0	0	0	0	-	-
30112-1	2	0	0	0	0	0	0	0	0	-	-
30112-2	4	0.5	0	0	0	0	0	0	0	0.5	-
30112-3	7	0.5	0	0	0	0	0	0	0	0.5	-
30112-4	11	0.5	0	0	0	0	0	0	0	0.5	-
30112-5	13	1	0	0.5	0	0	0	0	0	1.5	-
30112-6	18	1	0.5	1	0.5	1	0	0	0	3.0	1.0
30112-7	20	2	1	2	0.5	2	0	0	0.5	6.5	1.5
30112-8	25	2	1	2	1	2	0	0	1	7.0	2.0
30112-9	28	2	1	2	1	2	0	0	1	7.0	2.0
30112-11	42	2	1	2	1	2	0	0	2	8.0	2.0
30112-12	56	2	2	2	1	2	0.5	0.5	2	8.0	4.0
30112-13	85	2	2	2	1	2	1	1	2	8.0	5.0
30112-14	182	2	2	2	2	2	2	2	2	8.0	8.0

Table 1.

Quantitation of a single seroconversion series showing evolution of each HIV antigen and the sums of Early (Red) and Late (Green) reactivity at various times after first RNA detection.

Figure 2.

Panel A. Average intensities of each Western Blot antigen for 20 seroconversion series, showing similar distributions for Early (Red) and Late (Green Bands). Panel B. Sums of Early Reactivity (Red) and Late Reactivity (Green) used to generate a Western Blot Index for relating time post infection to sums of antigen intensities.



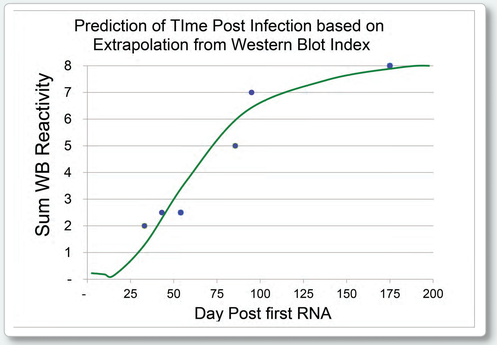


Figure 3.

A Western Blot Index based on the average antigen intensities of 20 seroconversion series (Green Line) was used to estimate time of infection of 4 additional individuals (blue squares) with known time of infection based on their Western Blot profile. The Index accurately predicted time of Infection to within 2 weeks of the time recorded for first RNA detection in these individuals.

CONCLUSIONS

Data from RV217 which allowed accurate establishment of time of first infection, was used to evaluate antibody reactivity to individual HIV antigens as detected on standard Western Blots at various times post known time of infection (Seroconversion series).

Antibodies to p24, p40, p55, and gp160 (Early Bands) develop quickly after infection, while more slowly evolving p31, gp41, p68, gp120 bands appear later in infection (75-85 days) and continue to increase in intensity past 180 days post infection.

A Western Blot Index based on average antigen intensities of 20 seroconversion series was generated and used to predict time of infection in other individuals from this study based on their Western blot profiles

The Western Blot Index accurately distinguishes between new, well established, or advanced infection and provides a useful estimate of the time of HIV infection up to 200 days from first infection.

Since Western Blots are already readily available and frequently used for confirmation of HIV infection, this approach offers a convenient measure of recency of infection that can be used in any standard clinical laboratory.

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The views expressed are those of the presenter and should not be construed to represent the positions of the U.S. Army or Department of Defense