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Seroincidence of Recent Human Immunodeficiency Virus Type 1 Infections in China▽

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A subtype B, E, and D immunoglobulin G capture immunoassay shows promise as a tool for estimating human immunodeficiency virus type 1 seroincidence from cross-sectional surveys, but the test-specific limitations suggest that an adjustment is necessary, and further validation of the assay with populations with divergent subtypes is needed.

Knowledge of human immunodeficiency virus (HIV) seroincidence could help in distinguishing between recent and long-term HIV infections, identifying recent trends or "hot spots," guiding better-informed prevention, care, and treatment, and determining areas or populations that are appropriate for vaccine, microbicide, and other prevention clinical trials; therefore, measuring HIV seroincidence has become increasingly important. However, the seroincidence is difficult to measure, and this has traditionally relied on the prospective testing and longitudinal follow-up of people at risk (2, 9, 20, 24). Estimation of the incidence by prospective studies faces challenges of bias, logistics, and high cost (2, 17). Laboratory assays to determine recent infection, such as p24 or HIV-1 RNA assays, require screening of large numbers of seronegative individuals to identify those in the very short "window period" (2). The less-sensitive enzyme immunoassays (EIAs) for detection of recent HIV type 1 (HIV-1) infection using cross-sectional surveys were designed based only on subtype B antigens (15, 18, 21, 26). An HIV-1 subtypes B, E, and D immunoglobulin G capture enzyme immunoassay (BED-CEIA) overcomes the disadvantages of less-sensitive EIAs and is designed to detect various HIV-1 subtypes (4, 12, 22, 23). Data demonstrated that this assay was useful for estimating seroincidence from a range of different HIV-1 subtypes using cross-sectional surveys (3, 6, 10, 13, 14, 15a, 19, 25; E. Karita, O. Manigart, G. Stevens, S. Allen, E. Hunter, M. Price, P. Fast, W. Stevens, N. Ketter, and the Rwanda-Zambia HIV Research Project, presented at the AIDS Vaccine Conference, Montréal, Canada, 6 to 9 September 2005). BED-CEIA has not been validated in China, where many disparate HIV-1 subtypes are circulating, including A, B, B′ (Thai B), C, D, F, G, circulating recombinant form 01_AE (CRF01_AE), CRF07_BC, and CRF08_BC (27). Therefore, we evaluated the performance of BED-CEIA among injection drug users (IDUs) in China.

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A total of 1,955 serospecimens from IDUs who tested HIV-1 seropositive from 2002 to 2005 were tested using the BED-CEIA. The 1,955 specimens included 29 from known seroconverters identified by weekly EIA (Kinghawk, Beijing, China) and reverse transcription-PCR (HIV–1/2 antibody assay; Kinghawk, Beijing, China) tests and 300 seropositive specimens from persons known to be infected for ≥2 years. All seropositive specimens were prescreened by EIA (Vironostica HIV-Uni-Form II plus 0; BioMerieux) and confirmed with Western blotting (HIV Blot 2.2; Genelabs Diagnostics). $CD4^+$ cell counts were determined using flow cytometric analysis (FACScan, Becton-Dickinson, Heidelberg, Germany) (5, 8). We performed BED-CEIA (Calypte Biomedical Corporation, Rockville, MD) using the algorithm of a single screening test and triple confirmatory tests to determine recent seroconversions. (11, 16, 19, 22). We calculated the cumulative, annualized incidence observed in the HIV Prevention Trials Network (HPTN) 033 Xinjiang cohort (unpublished data) by dividing the number of seroconversions ($n = 29$) by person-years. A 95% confidence interval was calculated for the observed incidence, assuming a Poisson distribution. In addition, a cross-sectional study randomly recruited 1,170 participants concurrently with the HPTN033 Xinjiang cohort amid the same IDU population; HIV positivity was determined by EIA screening test and confirmed by Western blotting. The BED-CEIA HIV-1 incidence was calculated as follows: $I = [(365/W)\,\mathrm{Ninc}]/[\mathrm{Nneg} + (365/W)\,\mathrm{Ninc}/2] \times 100$, where Ninc is the number of recent infections as determined by BED-CEIA, Nneg is the total number of HIV-seronegative subjects, and W is the mean window period of the assay, assumed to be 153 days (6, 16, 17,

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TABLE 1. Incidence of HIV infection among IDUs in Xinjiang Autonomous Regions, China, in 2002

Method	No. of participants	No. HIV+[a]	No. BED-CEIA+[b]	% HIV-1 incidence (95% CI)[c]
Cross-sectional survey[d]	1,170	225	34	8.2 (5.9–11.5)
Cohort study (HPTN033 Xinjiang cohort)[e]	475	42		8.8 (6.4–12.0)

[a] Number of participants whose serum tested positive in an EIA, with confirmation with Western blotting.
[b] Number of participants whose results were categorized as recent infections by BED-CEIA.
[c] 95% CI, 95% confidence interval. For the cross-sectional survey, the estimated incidence is a rate, and we applied the natural log scale transformation to compute variances and calculate the 95% CI for the incidence estimate as exp(log[*I*] ± 1.96/SQRT [Ninc]) (1). For the HPTN033 Xinjiang cohort study, HIV-1 incidence is the cumulative, annualized observed incidence, expressed as the number of new infections per 100 person-years.
[d] A cross-sectional study randomly recruited 1,170 participants concurrently with the HPTN033 Xinjiang Cohort amid the same IDU population; 225 of the 1,170 participants were proved to be HIV positive by the EIA screening test, confirmed by Western blotting.
[e] HIV Prevention Trials Network 033 (HPTN033) is a prospective cohort study. Participating IDUs were enrolled with 6 months of accrual and two semiannual follow-up visits. Participants were screened for seroconversion every 6 months.

22). The estimated incidence is a rate, and we applied the natural log scale transformation to compute variances and calculate the 95% confidence interval for the incidence estimate as exp(log[*I*] ± 1.96/SQRT [Ninc]), where SQRT indicates the square root operation (1).

There were five major findings. (i) There were no BED-CEIA false negatives for the 29 recently infected seroconversion specimens. (ii) Overall, 6.6% were false positive by BED-CEIA among 300 long-term infections. Though not statistically significant, a higher percentage of false positives was indicated among IDUs with $CD4^+$ cell counts of less than 50 than among those with counts of 50 or greater (12.0% versus 5.6%; $P = 0.1$). (iii) The BED-CEIA estimated and observed incidences for the HPTN033 Xinjiang cohort were similar (Table 1). (iv) In province "A" (Table 2), the BED-CEIA incidence was higher in the counties with higher HIV prevalence rates, and the incidence was lower in counties with lower HIV prevalence. (v) BED-CEIA proved to be a stable assay with minimal interrun, intrarun, and interoperator variation ($r^2 \approx 0.96$).

No BED-CEIA false negative for 29 specimens from subjects with known recent seroconversions was found. The BED-CEIA estimated incidence and observed (true) incidence agreed with each other. The reproducibility of BED-CEIA is high, which is consistent with previous findings (11). Although many disparate HIV-1 subtypes are circulating in China, subtype B′/B and C are predominant among IDUs in China; all specimens in our study were collected among IDUs. Therefore, it is plausible that the BED-CEIA could provide rapid and relatively inexpensive measurement for HIV-1 incidence among IDUs in China.

BED-CEIA falsely indicated (i.e., false-positive rate) that 6.6% of IDUs who had been infected for ≥2 years were recent seroconverters. This 6.6% BED-CEIA false-positive rate could lead to an overestimation of the HIV-1 incidence if there were a high proportion of persons infected for ≥2 years in the cross-sectional sample. We did not adjust for the BED-CEIA misclassification in our incidence estimate, because we did not know the proportion of the persons infected for ≥2 years in the pool of cross-sectionally obtained serospecimens. Limitations of our study also include our inability to characterize the specificity of results for those infected for 1 to 2 years. Thus, modeling is needed to adjust for the misclassification of individuals with long-term infection falsely identified as recent infections. Further validation of this assay with additional Chinese and other populations with divergent subtypes is needed.

TABLE 2. Incidence of HIV-1 infection among IDUs in five counties of province A, China, in 2004

County	No. of participants	No. HIV+[a]	% HIV prevalence % (95% CI[b])	BED-CEIA+[c]	% HIV-1 incidence (95% CI[b])
A	2,570	446	17.4 (15.9–18.8)	40	4.4 (3.2–6.0)
B	7,307	825	11.3 (10.6–12.0)	116	4.2 (3.5–5.0)
C	1,246	103	8.3 (6.7–9.8)	8	1.7 (0.9–3.4)
D	2,001	10	0.5 (0.2–0.8)	5	0.6 (0.2–1.4)
E	2,272	17	0.7 (0.4–1.1)	4	0.4 (0.2–1.0)

[a] Number of participants whose serum tested positive in an EIA, confirmed with Western blotting.
[b] 95% CI, 95% confidence interval.
[c] Number of participants whose results were categorized as recent infections by BED-CEIA.

We have presented the first step in validating the use of the BED-CEIA in China to identify recent infections. Definitive validation of the BED-CEIA should use larger panels of seroconverters from geographic areas where different subtypes are circulating. Cohorts should include persons with both early and late HIV infections and should be accompanied by epidemiological data to permit the exploration of reasons for false positives (e.g., low CD^+ cell counts, HIV antiretroviral therapy, and treatment of hepatitis B). The BED-CEIA shows promise for measurement of HIV-1 incidence by cross-sectional surveys in China, where the vastness of the country and the wide diversity of HIV transmission make targeted interventions for the areas and subpopulations of greatest need a more cost-efficient disease control strategy.

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