

Comparison of HIV incidence and risk factors for recent infection based on longitudinal follow-up versus cross-sectional cBED assay testing: a study in rural South Africa



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BACKGROUND

- HIV incidence estimates are important to understand the dynamics of the HIV epidemic and to target and evaluate interventions to prevent HIV infection. HIV incidence estimates can be obtained through repeated HIV testing of individuals in longitudinal surveillance (which is costly and complex), modelling using information of changes in HIV prevalence over time (which requires making uncertain assumptions, such as about HIV+ and HIV- mortality), or using laboratory tests which distinguish recent from non-recent HIV infections.
- One method to differentiate recent from non-recent infections uses the BED IgG-Capture Enzyme Immunoassay (cBED assay), which measures the proportion of HIV-1-specific IgG out of total IgG. This proportion increases with time after HIV seroconversion. Seropositive individuals who test below a certain threshold of this proportion (the BED threshold) are classified as recently infected, while those testing above the BED threshold are classified as non-recently infected. The time period following seroconversion after which infections are no longer considered to be recent (the so-called window period of the cBED assay) is usually estimated at approximately half a year [1,2].
- The cBED assay has been used to estimate HIV incidence in many countries, including in Ethiopia, Rwanda, South Africa, Uganda, Zambia, Zimbabwe, China, and the United States. However, there has been concern that the cBED assay-based methods overestimate HIV incidence to an unknown extent because some non-recent infections are classified as recent [3]. In some individuals (so-called non-progressors) the proportion of HIV-1-specific IgG never rises above the recency threshold, and in other individuals (so-called regressors) who have been HIV-infected for a long time, the proportion may fall below the threshold after having previously progressed above it (e.g. in people on ART, with current infection or AIDS).
- From previous empirical observations, it is known that the maximum progression time from seroconversion to a cBED value above the BED threshold is of the order of one year [1,2]. The fraction of all people who have been HIV-infected at least as long as the maximum BED progression time who are below the BED threshold is the long-term false-positive ratio (long-term FPR or ϵ).
- We use data from a large population-based longitudinal HIV surveillance to measure the long-term FPR in a rural African community with high HIV prevalence and HIV incidence [4], and then compare HIV incidence estimates based on the cBED assay to estimates based on longitudinal HIV surveillance.

METHODS

- The HIV surveillance area is located near the market town of Mtubatuba in the Umkhanyakude district of KwaZulu-Natal. All women aged 15–49 years and all men aged 15–54 years who were resident in the surveillance area at the time of visit of an HIV surveillance fieldworker were eligible for HIV testing.
- The samples for estimation of the long-term FPR consisted of cBED assay results for blood specimens contributed by individuals who tested HIV positive in the surveillance in the time period from June 2003 through June 2006. In order to be included in the sample, the specimens had to meet the following criteria. First, they were follow-up specimens from individuals who had previously tested HIV-positive in the surveillance. Second, the time period between the first positive HIV test and the follow-up specimen exceeded the maximum BED progression time. Third, the specimen was the earliest follow-up specimen that met the second criterion. Our count of long-term false-positive individuals included all individuals who were classified as recently HIV infected and had been infected for longer than the maximum BED progression time (number of specimens = 1,065 for BED progression time of 306 days). For the HIV incidence estimation based on longitudinal HIV status information, we included all individuals who tested at least twice for HIV in the period from June 2003 through June 2006 and whose first HIV test in this period was negative (4,869 individuals observed over 7,685 person-years, 224 seroconversions). For the cross-sectional cBED-based HIV incidence estimation, we used the first available HIV test for all individuals tested in the time period January 2005 through June 2006 (11,755 individuals).
- We implemented a simplified version of the McDougal formula [1] to estimate HIV incidence based on cBED assay information:

$$\hat{I} = \frac{fR}{fR + \omega N} \quad \text{with adjustment factor } f = [(R/P) - \epsilon]/(R/P(1 - \epsilon))$$

where R is the number of people who were classified as recently HIV-infected by the cBED assay, N is the number of individuals who tested HIV-negative, ω is the mean period of time from initial seroconversion to reaching the BED threshold, P is the total number of people who tested HIV-positive, and ϵ is the long-term FPR [5, 6].

- To control for differences in the sex-age composition between the sample used in the longitudinal HIV incidence estimation and the sample used in the cBED assay-based estimation, we weighted the sex- and five-year age group-specific longitudinal mean incidence rates by the proportions of individuals in each of the sex-age groups in the sample used for the cBED assay-based estimation [7].
- HIV status was determined by antibody testing with a broad-based HIV-1/HIV-2 enzyme-linked immunosorbent assay (ELISA; Vironostika, Organon Teknika, Boxtel, the Netherlands) followed by a confirmatory ELISA (GAC-ELISA; Abbott, Abbott Park, Illinois, USA). If HIV-positive status was confirmed, we used another spot from the same filter paper as used for the initial test in order to conduct the cBED assay (cEIA; Calypte © HIV-1 BED Incidence EIA, Calypte Biomedical Corporation, Maryland, USA).
- We compared associations between potential risk factors of HIV acquisition as found in multivariable Cox regression, using the data from the longitudinal follow-up, and multivariable logistic regression, using the data from cBED assay testing.

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RESULTS

- Using a maximum BED progression time of 306 days before the date of the cBED assay-tested specimen, we obtained a long-term FPR of 0.0169 (95% confidence interval (CI) 0.0100–0.0266). When we varied the length of the maximum BED progression time from 250 to 400 days (in daily intervals), we found that the estimate of the long-term FPR did not change significantly over the time interval, with minimum and maximum long-term FPRs of 0.0164 (95% CI 0.0097–0.0257) and 0.0190 (95% CI 0.0104–0.0317), respectively (Table 1) [8].
- Table 2 shows HIV incidence based on longitudinal follow-up and based on information from cBED assay testing (using the long-term FPR measured in this study, assuming a maximum BED progression time of 306 days, as well as a long-term FPR of 0.0560 based on other studies [1, 2]) [8].
- Assessment of potential risk factors of HIV acquisition yielded similar results for a range of variables (sex, age, urban vs. rural residence, and migration status) if analysis used the longitudinal information on HIV seroconversion and information from cross-sectional cBED assay testing.

| Maximum BED progression time | Sample size (individuals) | Number of individuals with false-positive cBED assay results | Long-term FPR | |
|------------------------------|---------------------------|--|---------------|----------------------|
| | | | Mean | 95% CI |
| (in days) | (individuals) | (individuals) | Mean | 95% CI |
| 250 | 1100 | 18 | 0.0164 | 0.0097-0.0257 |
| 260 | 1094 | 18 | 0.0165 | 0.0098-0.0259 |
| 270 | 1090 | 18 | 0.0165 | 0.0098-0.0260 |
| 280 | 1083 | 18 | 0.0166 | 0.0099-0.0261 |
| 290 | 1081 | 18 | 0.0167 | 0.0099-0.0262 |
| 300 | 1070 | 18 | 0.0168 | 0.0100-0.0265 |
| 306 | 1065 | 18 | 0.0169 | 0.0100-0.0266 |
| 310 | 1056 | 18 | 0.0170 | 0.0101-0.0268 |
| 320 | 1043 | 18 | 0.0173 | 0.0103-0.0271 |
| 330 | 1035 | 18 | 0.0174 | 0.0103-0.0273 |
| 340 | 1017 | 18 | 0.0177 | 0.0105-0.0278 |
| 350 | 991 | 17 | 0.0172 | 0.0100-0.0273 |
| 360 | 936 | 17 | 0.0182 | 0.0106-0.0289 |
| 370 | 818 | 14 | 0.0171 | 0.0094-0.0285 |
| 374 | 789 | 14 | 0.0177 | 0.0097-0.0296 |
| 380 | 773 | 14 | 0.0181 | 0.0099-0.0302 |
| 390 | 755 | 14 | 0.0185 | 0.0102-0.0309 |
| 400 | 737 | 14 | 0.0190 | 0.0104-0.0317 |

FPR = false-positive ratio, CI = confidence interval. Row in bold font shows FPR at twice the window period of 153, 180, and 187 days, respectively.

Table 1: Long term FPR (ϵ)

| | Estimation type | HIV incidence | |
|---|--|---------------|-----------|
| | | Mean | 95% CI |
| | Longitudinal measurement (per 100 people per year) | | |
| Crude | | 2.87 | 2.53-3.27 |
| Sex-age adjusted | | 3.09 | 2.69-3.52 |
| | cBED assay measurement (per 100 people per year) | | |
| Using long-term FPR $\epsilon = 0.0169$ | | 3.12 | 2.51-3.73 |
| Using long-term FPR $\epsilon = 0.0560$ | | 0.65 | 0.00-1.32 |

CI = confidence interval, FPR = false-positive ratio

Table 2: HIV incidence

DISCUSSION

- In a rural community in South Africa with high HIV prevalence, the long-term FPR of the cBED assay is substantially lower than previous estimates, which were around five percent [1, 2].
- Our study demonstrates that without a locally measured long-term FPR HIV incidence estimates based on the cBED assay may be severely biased, but that the cBED assay performs well in HIV incidence estimation, if a locally appropriate long-term FPR is used.
- We thus confirm the previous results by McDougal et al. [1] and Hargrove et al. [2] that cBED assay-based HIV incidence estimates are not significantly different from longitudinally measured HIV incidence, when a locally calibrated long-term FPR ratio is used to adjust for the imperfect long-term specificity of the cBED assay. At the same time, we have shown for the first time that the long-term FPR differs significantly across settings.
- The promise of the cBED assay for HIV surveillance, program evaluation and policy making, lies in the fact that it allows HIV incidence estimation from cross-sectional samples. Cross-sectional HIV status information, however, does not permit estimation of the long-term FPR, requiring researchers to obtain this parameter independently. It is thus important that the parameters necessary for HIV incidence estimation are calibrated using data from those settings where longitudinal follow-up is available.
- A meta-analysis of the long-term FPR of the cBED assay may help explain why the parameter estimates differ and allow the determination of valid regional parameter estimates.
- An alternative to using the long-term FPR in order to adjust cBED assay-based HIV incidence estimates for the presence of people who are falsely classified as recently HIV-infected is to use additional information on time since seroconversion to identify these individuals and correct the misclassification, such as laboratory parameters (CD4 count, total lymphocyte count, or viral load), clinical assessment, and screening for ART.
- cBED assay-based information can be used to investigate risk factors of HIV acquisition, as has been done in other settings [9].

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