eAppendix

HIV incidence estimation using the BED capture enzyme immunoassay: Systematic review and sensitivity analysis

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Basic description of the BED assay

The BED capture enzyme immunoassay (BED assay) was developed by researchers at the US Centers for Disease Control and Prevention (CDC) for the purpose of identifying recently acquired HIV-1 infections regardless of viral subtype.\(^1\) This was accomplished by producing a class-specific IgG antibody capture enzyme immunoassay (EIA) based on a trimeric branched peptide that includes gp41 immunodominant sequences from HIV-1 subtypes B, E and D – hence the name. The BED assay reports the proportion of HIV-1-specific immunoglobulin G (IgG) in total IgG as an optical density (OD) from spectrophotometer measurements. To minimize the variations that occur in different runs, a normalized OD (OD-n) is determined using a calibrator specimen.\(^1\) The proportion of HIV-1-specific IgG (and thus OD-n) increases with time after HIV infection.

Two major health organizations, the CDC and the United Nations Program on AIDS (UNAIDS), have issued statements regarding the use of the capture BED assay. While endorsing the assay for use in the US, the CDC recommendation\(^2\) lists several situations in which the assay can produce false-recent results (ie non-recently infected individuals that are falsely classified as recently infected), including advanced HIV disease, chronic co-infection, and antiretroviral therapy. The statement concludes that “the BED HIV-1 Capture EIA was developed for and is solely used in the US in the context of HIV surveillance” and that the assay “may be less successful in a specimen-based system where [...] critical data cannot be ascertained.” The most recent UNAIDS recommendation\(^3\) concluded that “the BED-assay captures not only recent infections, but also late stage HIV infection (with or without antiretroviral therapy)” and that “[t]here is evidence that assay characteristics vary by HIV-1 subtype.” UNAIDS thus called for “more research on the validity of the BED assay for estimating incidence.”\(^3\) Neither the CDC nor UNAIDS have commented on the use of the assay since 2007.
Literature search strategy

We carried out a systematic literature search in the PubMed electronic database. To identify articles, we combined search themes using the Boolean operators “and” and “or”: HIV “and” (BED assay “or” recent infection). Wherever possible, we drew search terms for each theme from the Medical Subject Headings (MeSH), the controlled vocabulary used for subject indexing in PubMed:


AND


We used all MeSH terms in their “exploded” versions so that all narrower terms categorized below each selected term in the vocabulary hierarchies were also included in the searches. In addition, we searched for terms that did not exist in MeSH using the “All Fields” category of the PubMed electronic database.

The development of the BED assay was first described by Parekh et al1 in a publication dated March of 2002. To ensure that we included all articles describing studies using the assay, we searched for articles published on or after the 1st of March 2000, ie two years prior to the publication describing the development. Our search period ended on the 4th of March 2009. In addition to PubMed, we searched the websites of the Conference on Retroviruses and Opportunistic Infections (CROI) (covering all CROI from January 1997 to March 2009)6 and the International AIDS Society (IAS) (covering all Conferences on HIV Pathogenesis and Treatment, and all International AIDS Conferences (AIDS) from 2001 through 2008)7 for abstracts containing the terms “BED”, “cBED”, “CEIA”, “EIA”,
“immunoglobulin G”, “IgG immunoassay”, “recency” and “recent infection”. These terms were also used to search the National Library of Medicine Gateway (NLM Gateway), which includes abstracts from twenty-nine HIV-related conferences. We further searched the reference lists of reviews, editorials, commentaries and all publications included in the final review. Finally, we asked colleagues with a research interest in HIV epidemiology or prevention to identify studies that report findings based on BED assay surveys.

Our initial PubMed search identified a total of 1181 unique studies, 1138 of which were excluded based on titles or abstracts. Studies were excluded at this stage if they did not report HIV incidence estimates, reported only HIV incidence estimates that were not based on BED assay surveys, or did not specify the populations in which a BED assay-based HIV incidence estimates were obtained. Studies were further excluded if they were not written in English, or were reviews, letters, editorials or commentaries. We conducted full-text reviews of the forty-three remaining publications and twenty-three studies identified in conference abstract databases, in NLM Gateway, or by colleagues. The only reason for exclusion after full-text review was that studies reported only HIV incidence estimates that were not based on BED assay surveys. We did not identify any studies through screening of references that were not also identified in one of the other searches. Four conference abstracts were excluded because they reported data contained either in a full-text article or in an abstract with a later publication date.

**Descriptive Results**

**Location, population setting, study type and assay**

Thirty-nine relevant studies were published between 2003 and 2009 (twenty full-text articles and nineteen abstracts) (Figure 2 and Table 1). In these studies, the BED assay was used to assess HIV incidence in regions throughout the world. Seventeen studies used data from Asia, twelve from Africa, six from North America, two from South America, and two from Europe. One study used data from more than one
geographical region. Eleven studies were conducted in the general population, seven in intravenous drug users, six in antenatal care (ANC) attendees, five in commercial sex workers (CSW) or female sex workers, five in men who have sex with men (MSM), three in the military, three in sexually transmitted disease (STD) clinic attendees, and one each in post-partum mothers, non-intravenous drug users, discordant couples, blood donors, fishermen, and police. Six studies used data from two or more different populations; they are included in the counts of each population above. The countries in which the reviewed studies took place and the populations in which the BED assay surveys were conducted are shown in Figure 3. The number of published studies using the BED assay to estimate HIV incidence increased from one in 2003, to three in 2004, four in 2005, six in 2006, and fourteen in 2007, but decreased slightly to eleven in 2008 (Table 1).

The BED assay was applied to samples collected as part of case-reporting surveillance (ie passive surveillance through which all individuals who are diagnosed as HIV-infected in voluntary counseling and testing centers are reported to a central organization, such as the US CDC), longitudinal population-based surveillance (ie active surveillance in which eligible individuals contribute blood samples for an HIV test repeatedly over time), sentinel surveillance (ie active surveillance that collects blood samples for HIV tests from all individuals belonging to a certain population group, eg individuals attending one of a selected set of antenatal care clinics), clinical cohort studies, preparatory studies for clinical trials, cross-sectional HIV surveys, and stand-alone HIV incidence studies.

Total sample sizes in the reviewed studies ranged from 400 to 87,178 across the 33 studies that reported the sample size (Table 1). Nineteen studies reported using the commercially available BED immunoassay produced by Calypte Biomedical Corporation, while the rest did not report the manufacturer of the assay used.
Incidence estimation approach

Fourteen studies\textsuperscript{10,11,17-20,32,33,35,40,41,43,47,48} used only a first-generation approach, eight\textsuperscript{14,23,30,31,34,37,42,44} used only a second-generation approach; one\textsuperscript{36} used second- and third-generation approaches; and two\textsuperscript{28,29} used formulae from all three generations. Fourteen studies\textsuperscript{12-16,21,24-27,38,39,45,46} did not report the formula used to estimate HIV incidence.

Three studies collected additional clinical information on study participants but did not consider using it to exclude or reclassify individuals.\textsuperscript{19,29,48} Three other studies indicated that their samples were unlikely to include individuals who could be falsely classified as recently infected. McDougal et al\textsuperscript{42} used “specimens largely derived from early infection,” which were known not to include individuals with AIDS symptoms or on ART treatment. Bärnighausen et al\textsuperscript{36} and Mermin et al\textsuperscript{30} did not apply inclusion criteria, but indicated that ART roll-out was not widespread at the time of the study.

Four studies used additional clinical information to reclassify or exclude individuals from the sample for BED assay testing. Three studies used a previous positive HIV test,\textsuperscript{39,41,43} two studies used ART status,\textsuperscript{39,43} and two studies used AIDS diagnosis.\textsuperscript{20,39} Buchacz et al\textsuperscript{43} excluded individuals from their sample who were both classified as recent by the BED assay and identified as having long-standing infection by additional information. Priddy et al\textsuperscript{41} and Jiang et al\textsuperscript{20} excluded all individuals identified as having long-standing infection by additional information, independent of BED assay test results. Hall et al\textsuperscript{39} did not exclude any individuals from the sample but classified all individuals as non-recent who were identified as having long-standing infection, independent of their BED assay test results. Li et al\textsuperscript{23} collected information on ART and AIDS diagnosis, but found that none of their study participants needed to be excluded or reclassified on the basis of this information. None of the other studies reported using extra clinical information for reclassification or exclusion from the sample for BED testing.

In addition to the two strategies described above to correct for BED assay imperfection, a few studies adjusted for selective HIV study participation. An important example is the
study of Hall et al\textsuperscript{39} which used a method developed by Karon et al\textsuperscript{50} to estimate the annual number of recently infected individuals in the US based on the number of recently infected cases detected by the national case-reporting surveillance. This estimate was calculated by dividing the number of cases detected in the surveillance by an estimate of the probability of detection. We have not reviewed the remainder of these methods in detail because they are specific in their application to certain study designs rather than to the BED assay.

Finally, two approaches were used to deal with HIV-positive individuals that had missing information on recent infection (eg as a result of insufficient sample to allow BED assay testing after an initial HIV test). The first approach\textsuperscript{43,48} excludes these individuals from the incidence estimation sample. The second approach\textsuperscript{29,30,40} assumes that the proportion of recent infections in these individuals is the same as the proportion in HIV-infected individuals with known recent infection status, and adjusts the incidence estimate accordingly.

**Optical density cut-off and calibration parameters**

Twenty-three of the thirty-nine studies reported optical density cut-offs, with seventeen using a value of 0.8\textsuperscript{15,20,23-26,28-30,33,36,37,39,41-43,48} and six using a value of 1.0.\textsuperscript{10,11,17,22,32,40} One study used a value of 0.75 in addition to a value of 1.0.\textsuperscript{22}

Window periods used in the calculation of incidence varied from 153 to 187 days. Of the nineteen conference abstracts, eleven\textsuperscript{12-14,16,21,22,38,44-47} did not report the window period used, while four reported using a value of 153 days,\textsuperscript{18,24-26} two reported 155 days\textsuperscript{15,37} and two reported 180 days.\textsuperscript{17,27} The window periods reported in the full papers are presented in Table 2. To compute annual incidence, unit consistency demands that a window period specified in days must be converted to units of years before being used in the calculation of incidence. In Table 2 we also report the length of year factor (365 or 365.25) used in each study and the corresponding effective window period specified in units of years.
In addition to the window period, studies using a second-generation formula required estimates of sensitivity, short-term specificity and long-term specificity, while studies using third-generation formulae only required estimates of the false-recent rate. With the exception of one study \(^{36}\) that used a locally-valid long-term specificity, all the studies that used a second-generation formula used the sensitivity, short-term specificity and long-term specificity as reported by McDougal et al.\(^{42}\) Some studies did, however, use varying degrees of precision for these parameters as reported in Table 2. All three studies using third-generation approaches estimated a false-recent rate for the local setting where the BED assay was applied,\(^{28,29,36}\) although one of them (Karita et al\(^{29}\)) did not use this estimate when calculating incidence.

**Confidence interval calculation**

With the exception of Wasinrapee et al\(^{18}\) who computed CIs using a normal approximation based only on the number of BED recent classifications (hereafter BED recent counts), none of the conference abstracts indicated how CIs were calculated. Two of the full-text articles\(^{23,33}\) did not report how CIs were calculated, while eight\(^{20,29,30,32,35,41-43}\) used a normal approximation based only on the number of BED recent counts, one\(^{19}\) used a log transform of a normal approximation based only on BED recent counts, two\(^{31,34}\) used a normal approximation based on the BED recent and HIV-negative counts, one\(^{40}\) used a modified Wald method, two\(^{28,39}\) used a delta method, and one\(^{36}\) used a full trinomial distribution to approximate CIs. In all of these studies only uncertainty resulting from counting error was taken into account, while the remaining three papers\(^{10,11,48}\) additionally accounted for the uncertainty associated with the window period by using a Bonferroni procedure that combined the window period CI with the CI for the counting error that was calculated using a Poisson distribution.\(^{51}\) None of the studies using second- or third-generation approaches attempted to account for uncertainty stemming from error in the estimation of sensitivity and specificity parameters.
References


32. Sakarovitch C, Rouet F, Murphy G, Minga AK, Alioum A, Dabis F, Costagliola D, Salamon R, Parry JV, Barin F. Do tests devised to detect recent HIV-1 infection


42. McDougal JS, Parekh BS, Peterson ML, Branson BM, Dobbs T, Ackers M, Gurwitz M. Comparison of HIV type 1 incidence observed during longitudinal follow-up with
incidence estimated by cross-sectional analysis using the BED capture enzyme immunoassay. AIDS Res Hum Retroviruses 2006;22(10):945-52.


