ABIE Toolbox Version 3
(Tools for Assay-Based Incidence Estimation)

Overall Release Note
June 2015

Introduction to ABIE
SACEMA (sacema.org) is releasing a major update to its Assay-Based Incidence Estimation (ABIE) toolbox.

The central concern in ABIE is the estimation of (presumably HIV) incidence through cross-sectional biomarker measurement – in particular, biomarkers supporting classification of cases (infections) as recently or non-recently acquired. Although almost all attention paid to this topic is in the context of HIV epidemiology, the underlying concepts are in fact general, and they may, in time, find meaningful application to other chronic conditions.

This set of spreadsheets (“calculators”) is freely available in open source, editable form, and as such comes without warranty, although great care has been taken to ensure correctness and stability. Sheets are superficially locked to help prevent unintended edits, but can be unlocked without a password. They are distributed in Microsoft Excel (.xlsx) format. As they rely only on simple formulas (i.e. no embedded visual basic or complex macros) they can be easily ported to other platforms.

Potential users include:
- researchers planning, implementing, or analysing data from, major surveys
- officers in departments of health or statistical bureaux
- reviewers of protocols or articles
- product developers
- funders
- teachers/trainers and students

The lack of any formal warranty notwithstanding, users are invited to contact SACEMA for assistance and are indeed requested to provide any notification of possible errors, or feedback about functionality.
Overview of ABIE v3

June 2015 marks the release of version 3 of the ABIE toolbox.

In version 3, there are separate calculators (spreadsheet “workbooks”):

- **Incidence_Prevalence_Calculator** – produces a prevalence and incidence estimate from a single cross-sectional survey
- **Incidence_Difference_Calculator** – systematically compares incidence estimates from two cross-sectional surveys
- **Sample_Size_Calculator** – calculates a minimal sample size to provide desired precision of incidence estimate (in a hypothetical context) or “power” to detect a difference in incidence between two hypothetical contexts
- **Power_Calculator** – calculates “power” to detect a hypothetical incidence difference/trend
- **Test_Performance_Calculator** – summarises “performance” of a recent infection test (into a standard error of the incidence estimate), given estimated test properties and the prevalence/incidence in a hypothetical context

While each spreadsheet calculator tackles a narrow class of calculations, some spreadsheets enumerate various logically distinct cases, and offer multiple layers (“worksheets”) to provide relevant options, support sensitivity analysis, etc. Each ABIE worksheet level tool is organised around the following principles:

- All cells which are not designated for data entry are locked by default. They can be unlocked without a password using the “unprotect sheet” functionality on the “review” tab.
- Cells, and text, are colour coded to indicate whether they represent input, intermediate results, primary output, or ancillary results. A colour key is provided in each relevant worksheet.

Each calculator is accompanied by a non-technical “user guide”. The user guides for each calculator are included in a dedicated worksheet.

Little to no technical knowledge of the underlying statistical theory is required to use the calculators, but users need to be familiar with basic ideas around HIV epidemiology and the use of assays to establish recent vs non-recent HIV infection. A technical document detailing the new formulas derived for the development of ABIE v3 is in preparation.

To support correct use of the tool, there are numerous warnings and error messages designed to appear in response to a variety of triggers. These messages are usually located in a column a little to the right of a cell which triggered them. Within a given workbook, all of these messages are in a single column, though the column used varies between workbooks. The messages column is indicated near the top of each workbook, and it is worth checking that this column is visible while inputs are being provided.
• **Warnings** highlight possible problems such as input parameters that are unlikely to be consistent with robust use of the tool.

• **Errors** indicate a fundamental breakdown in consistency, such as a greater number of recent HIV infections than HIV infections. In the case of an error, expected output is suppressed.

Some sheets include dynamic charts intended to support interpretation of inputs and results. Some sheets are entirely comprised of a single chart, derived from data supplied in another sheet, usually in a self-evident fashion, as documented in the associated user guide. There may be an entire sheet of intermediate results to populate the figure – this intermediate results worksheet will be hidden by default, but can be made visible without a password (*home* tab, *cells toolbar*, *format button* -> drop down menu: *hide & unhide* -> secondary dropdown, *unhide sheet*).

**New Features**

This new release (version 3) implements a number of notable updates:

**User-specified “design effects”**

Users can now provide scale factors for variances which arise from multi-level (cluster) sampling (as opposed to independent individual level random sampling). There is provision for adjusting uncertainty in estimates of

1. the proportion of HIV infected individuals (i.e. prevalence) and
2. the proportion of “recent” results among HIV positive individuals tested for recent infection.

Additionally, the primary **Incidence/Prevalence Calculator**, which processes the proportions (of HIV infected, and “recently infected” individuals) obtained in a single cross-sectional survey, is also accompanied by a “global design effect” sensitivity analysis.

It should be noted that:

• There is no clear consensus on approach, and there are no mature tools, for estimating the design effect parameters for population based HIV surveys.

• The design effects implemented in ABIE v3 are not just two places in formulas into which a single “design effect” should be inserted. They are logically independent parameters, which capture the effects of statistically independent processes.

• The ABIE tools do not provide any functionality to derive or justify design effect estimates.

• It is not appropriate to scale the variances of the test property (MDRI and FRR) estimates, as these are not estimated in the incidence survey, but rather arise in the recency test development process.

**Improved functionality for comparing incidence estimates from two surveys**

There are some nuances around the interpretation of two (or more) cross-sectional recency-biomarker surveys, for example, to estimate an incidence trend in a population.
The assumptions around similarity of contexts for two surveys have been made more flexible. It is now possible to treat estimates of MDRI and FRR to be fully shared, or fully independent, between two surveys. Alternatively, MDRI estimates can be shared while FRR estimates are independent. Also, a more flexible null hypothesis has been implemented to calculate power and p values.

The incidence ratio test raises numerous subtleties in relation to the difference test, which are difficult to resolve within the limitations of a simple formula based spreadsheet, and so this feature is no longer explicitly implemented.

Consistent handling of incomplete “recency status” ascertainment
Previously, only the primary Incidence/Prevalence Calculator had the option of explicitly reporting how many of the HIV positive subjects had a successfully obtained recency test result (it should always be the vast majority). This option is now available in each calculator. The implementation of this “fix” should not be confused with some kind of non-trivial “multiple imputation” which is sometimes used to paper over simple missing data.

Improved nomenclature
Previous versions referred to “coefficient of variation” both as a user-provided measure of precision in estimates of such parameters as test properties (MDRI and FRR) and also as a spreadsheet-computed metric of precision of estimates of incidence. This is now referred to as “relative standard error”, in line with more conventional usage.

The wording associated with the Power Calculator has been modified to clarify the nature of the presumed test and the performance metric which is calculated, namely the probability of inferring an incidence difference of the correct sign, in a two-tailed test.

General warnings and error messages
Numerous new warning and error messages, in the spirit of those used in earlier versions, have been implemented to support the new features in this release, and some old messages have been modified.

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Citation
The official version 3 release of the ABIE tool set is found at: http://www.incidence-estimation.org/page/tools-for-incidence-from-biomarkers-for-recent-infection

When using ABIE v3 tools, please cite the source of the tools (the URL above) and the following article, which provides the theoretical foundations and derives the underlying formulae used:


The most significant details which are new in ABIE version 3, such as the use of design effects and the improvements to incidence estimate comparisons, are not published outside of the tools themselves, as of the release date (June 2015). They were derived by Alex Welte, Cari van Schalkwyk, Reshma Kassanjee and Simon Daniel. The tools themselves, including the URL above, should be cited.

Acknowledgements
The ABIE family of tools has been developed by (alphabetically): Hilmarie Brand, Simon Daniel, Reshma Kassanjee, Tom McWalter, Cari van Schalkwyk and Alex Welte.

Funding support for the development of SACEMA’s ABIE tools has historically been variously provided by the South African National Research Foundation, the Bill and Melinda Gates Foundation, the (erstwhile) Canadian International Development Agency, UNAIDS, and the World Health Organisation.

Many people have contributed indirectly to this work by providing feedback on major and minor aspects and revisions. We wish to thank, in particular, Meade Morgan, Stefano Ongarello and Eduard Grebe for their useful feedback on this version.

Further Reading

**A. Primary papers**

   A pioneering paper exposing the original concept of estimating HIV incidence by measuring the prevalence of a transient status of recent infection.

A landmark article elaborating on Brookmeyer and Quinn’s concept that describes the first serological incidence assay (a detuned version of an Elisa diagnostic test) and displaying its potential use.


A general theoretical framework for deriving biomarker-based incidence and estimator uncertainty accounting for the occurrence of false recent infection results. Comes after a decade of literature debating on how to adjust incidence estimates for laboratory “misclassification”.

**B. Examples of field applications of biomarker-based incidence**


A study comparing cross-sectional and longitudinal HIV incidence estimates and one of the first attempts to adjust the biomarker-based estimate for false recent results.


Using data from rural South Africa, the study compares BED assay based incidence estimates to longitudinal estimates. It points out the importance of an appropriate estimation of false-recent rate in the population of interest.


A study comparing BED assay based incidence estimates to longitudinal estimates in three cohorts in Thailand.

**C. Development and evaluation of incidence assays**


After Janssen’s paper, a report of the development of an improved standalone incidence assay (BED), later calibrated for and used in numerous worldwide field applications.


A study demonstrating the performance of the new LAg assay over BED and an older generation avidity assay in terms of consistency of properties in different populations and subtypes.

A study presenting an algorithm of laboratory measurements (MAA for multi-assay algorithm) resulting from a series of work trying to limit the false recent rate of single incidence assays.


This study takes advantage of the large CEPHIA specimen repository to evaluate properties of five available tests for recent infection used as single incidence assays. It confirms that all assays are (individually) affected by a large proportion of false recent results in ARV treated individuals.


Provides objective guidance criteria to maximize the properties of a test for recent HIV infection in order to estimate incidence. The paper makes a strong point in focusing on the precision of the final incidence estimator rather than thinking of the performance of a test in terms of sensitivity/specificity.

D. Reviews on biomarker-based incidence


A review of the general principles and uses of recent infection status to derive HIV incidence. Covers various types of applications.


A review covering available technology for detection of recent HIV infection.


A review of BED assay applications that explores sensitivity of incidence estimates to different formulation of the estimator (incidence formulae) and different calibration parameters of the assay. With an interesting focus on good practices for reporting results.

15. WHO. When and how to use assays for recent infection to estimate HIV incidence at a population level. 2010 Mar p. 1–60.

A guidance document summarizing practical aspects of field application of a biomarker-based cross-sectional incidence study, with examples.


A review of current effort and strategy of international groups and institutions to promote reliable incidence assays.

**E. On other HIV incidence estimation methods**


A review of existing methods and remaining challenges to estimate HIV incidence (and prevalence).


A report of latest development of WHO/UNAIDS modelling tools fitting prevalence data from HIV surveillance systems.


The study evaluates by numerical simulations a new modelling approach that uses (individual) prevalence data and mortality rates to estimate HIV incidence.

**F. On laboratory-based estimation using case-based surveillance data**


A landmark paper providing and evaluating by numerical simulations a new model to estimate HIV incidence (yearly number of new infections) based on recent infection status among new diagnoses reported in case-based surveillance.


Application in the US of Karon et al’s model based on recent infection testing plus a back-calculation model provide two independent HIV incidence estimates at a national level.


Application in France of a Karon-like model to estimate HIV incidence rates in different subgroups at a national level.
Glossary
The suite of tools repeatedly uses some common or specialised (to this sub-field) terms which it may be prudent to define here. These are not detailed technical definitions, but are intended to serve as reminders, or, should they be unclear, would serve to highlight the need to investigate primary sources.

- **HIV infected individual**: The protocol-specific case definition for HIV infected individual needs to be very clearly understood. This is because there is no universal standard, although there have for some years been minor variants on protocol involving sensitive screening tests and highly specific “confirmatory” tests. This is shifting even for clinical practice, and is even more fluid for research settings, which may use viral nucleic acid and antigen detection, and not even need classical serology for confirmation of HIV infection.

- **Mean duration of recent infection (MDRI)**: The average time for which subjects satisfy a particular “recent infection” case definition, within a specified recency cut-off time T after (verifiable) infection (which is context/protocol specific).

- **False-recent rate (FRR)**: The (context specific) fraction of tests, performed on individuals infected for more than the time cut-off T, which produce a (false) recent result. This term has seen many variants. FRR is inspired by the long used term “error rate” to refer to the fraction of tests which fail in some sense. Note that there is fundamentally no such thing as a false non-recent result – the phenomenon that some individuals transition to the non-recent case definition at relatively early times post-infection, compared to the average time, is accounted for in the MDRI.

- **Incidence as a (or, an “instantaneous”) rate**: This is the most fundamental metric for expressing the rate at which HIV infections occur in the susceptible (aka “at risk”) population, and is naturally expressed as a number of (infection) events per person time at risk in the referenced susceptible population. In the case of some other epidemiological contexts (such as influenza) it is not uncommon to refer to person time in the entire population, rather than the susceptible sub-population. While, in principle, any unit of time may be used (days, weeks, months) the usual unit in HIV discourse is the year. The value of such a rate can in principle take any value, as it changes with choice of units in which time is measured.

- **Annual(ised) risk of infection**: It is also common to report the “cumulative” probability of infection over a specific period of time, such as one year. It is a subtle point, not worth expositing here in detail, that the instantaneous incidence, with time measured in years, is not, in principle, the same value as the annual risk of infection. Suffice to note that incidence can take any value for any period of time (depending on choice of units and varying risk factors) but the probability of infection cumulated over a particular time period is always a number between zero and one.

- **Recency time cut-off T (“Big T”)**: On account of the fact that it is possible for a recency test to classify some individuals as recently infected at long times post infection, the use of a time cut-off T has been introduced to assist in the housekeeping. The details of how this works are well beyond the scope of this user guide.
• **Relative standard error (RSE):** This widely used term refers to the ratio of the standard error of an estimate divided by the point estimate.

• **Null hypothesis:** A usually artificial assumption (not necessarily strongly believed, and perhaps strongly suspected to be false) which data either falsifies or fails to falsify.

• **p-value:** The probability, calculated under a particular null hypothesis, of seeing a specified deviation from a null value in a test-statistic under consideration. The classic p-value in this context answers the question: If the incidence were really the same in two populations which have been surveyed, what is the probability of seeing a point estimate of the incidence difference, the absolute value of which is at least as large as the one observed?

• **Significance:** This widely used term refers to a threshold on a p-value, below which the experimenters will reject a given null hypothesis.

• **Sample size:** The total number of individuals whose HIV status has been, or is proposed to be, assessed.

• **Design effect:** This parameter captures the impact of hierarchical (clustered) sampling, and reports the ratio of the actual variance of a metric (such as a prevalence of some defined case) to the variance that would be obtained for the same metric if the individuals surveyed had been drawn independently from a large population rather than from initially selected regions/clusters.