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A generalizable method for estimating duration of HIV infections using clinical testing history and HIV test results

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Abstract

Objective.—To determine the precision of new and established methods for estimating duration of HIV infection.

Design.—A retrospective analysis of HIV testing results from serial samples in commercially-available panels, taking advantage of extensive testing previously conducted on 53 seroconverters.

Methods.—We initially investigated four methods for estimating infection timing: 1) “Fiebig stages” based on test results from a single specimen; 2) an updated “4th gen” method similar to Fiebig stages but using antigen/antibody tests in place of the p24 antigen test; 3) modeling of “viral ramp-up” dynamics using quantitative HIV-1 viral load data from antibody-negative specimens; and 4) using detailed clinical testing history to define a plausible interval and best estimate of infection time. We then investigated a “two-step method” using data from both methods 3 and 4, allowing for test results to have come from specimens collected on different days.

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AUTHOR CONTRIBUTIONS

Conceptualization: CP, TP, SF, EG, AW, RK. Specimens and data: SM, SMO, KD, SF. Formal analysis: TP, CP, SF. Writing: CP, SF, TP. Review and editing: CP, SF, TP, KD, EG, SM, RK, MB, GM, SMO, AW.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification purposes only and does not constitute endorsement by the CDC or the US Department of Health and Human Services.

Results.—Fiebig and “4th gen” staging method estimates of time since detectable viremia had similar and modest correlation with observed data. Correlation of estimates from both new methods (3 and 4), and from a combination of these two (“2-step method”) was markedly improved and variability significantly reduced when compared with Fiebig estimates on the same specimens.

Conclusions.—The new “two-step” method more accurately estimates timing of infection and is intended to be generalizable to more situations in clinical medicine, research, and surveillance than previous methods. An online tool is now available that enables researchers/clinicians to input data related to method 4, and generate estimated dates of detectable infection.

INTRODUCTION

Biomarkers that change over time are often used to assess the chronicity or duration of an illness. When physicians “stage” cases of most infectious diseases (e.g., syphilis, hepatitis B, or Epstein-Barr virus infections), they use serologic assays to assess infection duration. Knowing if a case has very early or later-stage infection can help a physician interpret patient symptoms, inform the prognosis, and affect treatment and public health management. Unfortunately, while serologic staging is conceptually appealing, staging systems often provide very limited information in practice. One major issue is that staging systems are developed using specific assays, and thus require that those assays be used to yield valid estimates of infection duration for individuals. Even more important, test results often come from visits that are weeks or months apart, making it challenging to estimate infection timing. To assign a specific stage directly from a set of test results, one must also be looking at tests from specimens collected during the time serologies are evolving. This means infection staging can only be done during a brief window of time.

HIV infection staging has been approached in this manner, using a serologic staging rubric developed by Fiebig, et. al.¹ Originally proposed in 2003, this system describes five discrete, sequential stages of early infection with different HIV test result patterns. These stages are referenced in nearly all discussions related to acute HIV infection.² Unfortunately, the window of acute HIV infection is brief, and discrepant results indicating acute infection are rarely seen in clinical practice. Also, three of the five key assays used to define Fiebig stages—the HIV p24 antigen ELISA, HIV IgG antibody ELISA and HIV antibody Western blots—are classes of HIV test that are no longer available or commonly used. The types of tests available today (“4th generation” antigen/antibody HIV assays, IgM or IgG-antibody rapid tests, HIV-1/2 differentiation assays, and quantitative viral loads) were not part of the Fiebig analysis. Recently, Ananworanich and colleagues³ proposed a simplified system of three “4th gen stages” in which an antigen/antibody assay would function in place of the p24 Ag ELISA. To date, neither the staging system proposed by the Fiebig nor that of Anorworanich have been independently validated as a predictor of HIV infection timing.

The shortcomings of traditional serologic staging are germane in cases of HIV seroconversion or acute HIV infection, (i.e. after detectable HIV viremia but before the antibodies to HIV infection are present in detectable levels). In these situations there is urgency to identify HIV infection related to important prevention and clinical benefits. For

HIV prevention, the risk of HIV transmission to exposed infants and sexual partners is many times greater for index patients who are acutely or recently infected—and different interventions are thus often recommended for infants⁴ and for sexual partners⁵ determined to have been exposed to acute or recent HIV infection.^{6, 7} Clinically, ART initiated in the first two months after infection has been associated with reduced seeding of HIV reservoirs^{8, 9} and better HIV specific immune responses.¹⁰⁻¹² In randomized trials comparing immediate versus delayed ART for patients with recent (<3–6 month) HIV infection, immediate ART has resulted in improved HIV control and increased CD4 cell levels,¹³ and reduced long-term malignancy risk¹⁴ when compared to ART initiated after a delay. The sense of clinical urgency around acute HIV infections often drastically affects the way that diagnosing emergency room physicians, HIV testing counselors, and clinics respond to a new diagnosis of HIV—leading to urgent prioritization of partner management strategies and triggering rapid care navigation and immediate ART (prior to meeting a primary provider, and without waiting for a drug resistance result).¹⁵

Accurate, individual-level information on HIV infection timing is key to interpreting many research studies of HIV transmission, early disease pathogenesis, ART, and diagnostics. Further, the concept of infection time is central to global surveillance programs, which measure HIV incidence in part by classifying individuals in population surveys as being infected less than some average duration of time, as recently reviewed in Murphy *et al.*¹⁶

For prospective studies, as well as those using stored specimens and historical test results from many years ago, updated strategies for staging infection are needed. In the fourteen years since the Fiebig staging method was first published, investigators at the U.S. Centers for Disease Control and Prevention (CDC)¹⁷⁻¹⁹ and others^{3, 20-22} have reported additional experiments using seroconversion panels that have much more precisely described the observed distribution of test conversion times for many more assays. The CDC studies clearly estimate the median and range of times that elapse between individuals' first detectable HIV viremia and their conversion on nearly all other HIV tests.²³

We hypothesized that these test-specific “diagnostic delays” could be used to infer a range of plausible dates of first detectable viremia from any testing history—even when same-sample “acute infection staging” was not possible, and that this method should provide equally accurate estimates for historical or modern assays. We also hypothesized that quantitative viral load information would be as useful or more accurate than p24 or antigen/antibody assay information in assessing the timing of acute HIV infections detected prior to antibody seroconversion. In this paper, we describe methods based on these principles; demonstrate their performance and compare them with older methods (i.e. Fiebig and 4th gen staging models); and provide guidance including links to online tools for improved estimation of HIV infection duration.

METHODS

HIV test results including qualitative and quantitative HIV-1 viral load were obtained through testing of commercially available seroconversion panels from 53 individuals documented to have seroconverted to detectable HIV infection while participating in

programs for repeat blood plasma donation. We sought to identify and access all available data from plasma donor panels in which all or most of the Fiebig-era tests, as well as more modern tests, had been performed and results were available across a variety of timepoints. This included panels from CDC and a variety of commercial panels used in the original Fiebig analysis and other subsequent analyses. We selected panels for the analysis in which the last specimen where no RNA target was detected “Target not detected” (TND) or “negative”) or with an HIV viral load quantified <100 copies/mL on any assay, was followed within 7 days by at least one specimen with >100 copies/mL HIV RNA on any assay. HIV test results for each timepoint were analyzed including results that were publicly available for each specimen panel,^{24, 25} and additional results of re-testing of plasma aliquots by a variety of HIV diagnostic assays as these became available over time; more detail on these methods have been reported elsewhere.^{17-19, 23} As most of the panels used were commercially available, archival HIV testing data were available on each company’s website^{24, 25} and these were also included in relevant analyses.

In theory, the ideal reference standard for an infection dating scheme would be the infectious event itself; however, as no assay can measure the infectious event, there is a need to agree on an assay-based reference standard. We call this the date of detectable infection (DDI), defined operationally as a hypothetical date on which “ramp-up viremia” first exceeded 1 copy/mL of HIV RNA in plasma, or DDI_1 , likely several days after exposure.¹⁶ To estimate the actual date of infection, the average estimated duration of the eclipse phase²³ can be added to the estimate of days since DDI_1 for a specific subject. In any analysis, the threshold for DDI can be set to a value appropriate for viral detection during the ramp-up phase. For this paper, we used a modified reference standard for the specimen sets we examined, defining the date of detectable infection as the date at which viremia exceeded 100 copies/mL (the DDI_{100}). This particular threshold was chosen for consistency with previous papers¹ and to account for variability in assay detection limits in our retrospective, pooled dataset.

For each individual seroconverter’s specimen set, a date of detectable infection (DDI_{100}) was estimated as occurring between the last bleed at which HIV RNA was either TND or detected with <100 copies/mL, and the first bleed at which HIV RNA was detected 100 copies/mL. Within this (maximum 7 day) window, the exact date of crossing the 100-copy threshold was estimated for each seroconverter by linear interpolation of \log_{10} -viral load graphed vs. time. For this calculation, TND values were assigned half the detection limit of the most sensitive negative RNA assay used.

We initially investigated four methods (detailed in the Supplemental appendix) for estimating infection time: 1) using Fiebig stages;¹ 2) using the “4th gen” modified staging system;³ 3) using quantitative viral load results from antibody-negative specimens utilizing a linear mixed effects regression model of “viral ramp-up” dynamics with a random slope and intercept; and 4) using the details in the clinical testing history, including negative and positive assay results to define a plausible interval and best estimate of infection time. Examples of this testing history-based estimation procedure are illustrated in Figure 1, and more detail is available in Grebe *et al.* (forthcoming). We explored whether methods 3 or 4 performed at least as well as the widely-accepted Fiebig method, with greater flexibility and

applicability to today's currently available assays. After initial results indicated suitable performance of the viral ramp-up method (method 3), we lastly investigated a "two-step method" for evaluating the complete testing history. The two-step method was designed to incorporate information for people identified after viral ramp-up was complete and the antibody response had developed, which is the most common real-life application of these methods. The two-step method calculated infection date estimates on viral load information (method 3) when it was available on an antibody negative specimen, but otherwise used the rest of the subject's testing history in the calculation using method 4.

Statistical analysis.

All analyses presented in this paper concern only the specimen panels with a documented date of first detectable viremia at a 100-copy threshold (with results going from <100 to >100 copies RNA within less than 7 days). The DDI_{100} was determined for each seroconverter panel. The time in days since the DDI_{100} could then be directly calculated for each specimen in that panel.

For primary analyses and for sensitivity analyses in which assumptions and included data varied, the performance of each prediction model was assessed by comparing model-estimated time with the gold standard for DDI_{100} . Prediction errors were assessed by the root mean square error when comparing to a gold standard. Specifically, if X_i represents the gold standard estimate of the infection time for person i , and Y_i is the corresponding estimate derived by a method under consideration, then the overall root mean square deviation exhibited is

$$R_Y = \sqrt{\sum_{i=1}^N (X_i - Y_i)^2}$$

The standard errors were computed using bootstrap resampling. When comparing the accuracy of two methods which give estimates Y_i and Z_i for each patient, we computed the statistic $R_Y - R_Z$ and conducted hypothesis tests using the bootstrap confidence interval. We also used the intraclass correlation as a measure of agreement.

RESULTS

Data from 206 specimens (n) and 31 individual seroconverters (N) were obtained and considered eligible for inclusion in the final analyses, based on an interval 7 days between the last TND and first detectable result on a quantitative viral load. Of these, 14 individual series/99 specimens (N/n = 14/99) were represented in the 2003 Fiebig manuscript analysis ("Fiebig panels") while 30 individual series/107 specimens (N/n = 30/107) were represented in the CDC papers from which most of the offsets in Table S1 were derived (the "CDC panels"). Intervals between last specimen with HIV RNA <100 and first specimen with HIV RNA >100 ranged from 1 to 7 days with a mean of 3.5 days. The duration from DDI_{100} for specimens in the analysis ranged from 1 – 43 days. To preliminarily validate individual methods, the panel data used for original model development were excluded; i.e. data from Fiebig panels were excluded from the Fiebig method validation, and data from CDC panels were excluded from the testing history method validations. After this validation, we

performed a head-to-head comparison of the different candidate methods using the entire specimen set.

In the initial method validation, Fiebig method estimates of time since DDI₁₀₀ for observations with Fiebig stage I-V on CDC panels (N/n = 14/92) had a modest correlation with observed times in the original Fiebig data, with an intraclass correlation (ICC) of 0.671 (95% CI 0.542, 0.770) and a root mean square (RMS) error of 13.15 (95% CI 10.77, 15.87). The distribution of times associated with each Fiebig stage (including all panels for the head-to-head comparison) are shown in Table 1: times were generally very close to those expected in stages 1–4, with average error less than 7 days at each of these stages. Estimates of infection time for Fiebig stage V were much shorter than expected, perhaps due to truncation of sample collection in the plasma donor population. Estimates for the three “4th gen” stages gave similarly small errors (ICC of 0.779 and RMS error of 4.2). The Fiebig and 4th gen staging methods performed similarly in the final comparison using all staged specimens for each method, as shown in Table 2 and illustrated in Figure 2(A and B).

The two new methods developed for this paper were the acute viremia “step 1” model—applicable to antibody-negative specimens only—and the more generalizable “step 2” model computing a plausible interval and EDDI from all positive and negative results in the testing history. As shown in Table 2 and Figure 2C, both of these new methods performed better than complete Fiebig staging in head-to-head comparison using the same test result information and specimens. For the step 1 acute viremia model and for the combined, 2-step procedure (Figure 2D), correlation of estimates with actual infection time was markedly improved and errors significantly reduced when compared with Fiebig estimates on the same specimens (Table 2). By itself, the step 2 model gave performance that was similar to that of Fiebig and 4G staging when applied to discrepant results on same-day testing (see Table 2, **and** Figure 2C). However, the goal of including the new method was to improve flexibility over more traditional “staging” approaches—making it possible to use real world results, including results on newer assays and from multiple test dates during seroconversion. We therefore tested the sensitivity of the step 2 model to these situations in a series of scenarios where more or less information was included in the testing history model. As shown in Figure 3 **and** Table 3, the inclusion of information from two separate dates actually improved precision of estimates when compared with using the same system on same-day discrepant results. A scenario using newer assays (e.g. an antigen/antibody immunoassay, followed by the Bio-Rad Geenius™ HIV-1/2 Supplemental Assay) also gave significantly better performance than one using older tests (e.g. antibody only, IgM-sensitive immunoassay, and the Bio-Rad GS HIV-1 Western blot) on the same specimens (Table 3).

DISCUSSION

In this paper we demonstrate a novel method for estimating the timing of HIV infection that is more accurate than prior methods and much more likely to be applicable in the real world. Performance differences favoring the new method were generally small but statistically significant; whether or not such differences are meaningful in practice will depend on the clinical or research question being asked. We furthermore showed that estimates by the new method were robust to variation in the types of HIV test used, and were equally accurate

whether tests were included from specimens on the same day or from specimens taken on different days during seroconversion. The new system may thus overcome key limitations of previous methods. Up to now, acute infection staging has relied on successfully obtaining and testing an acute infection sample, and has required results from multiple different tests, some of which are no longer available. By using results from any two tests, even if obtained from specimens collected on different days, the new method is intended to be generalizable to many more situations in clinical medicine, research and surveillance.

One of the key features distinguishing the current method is its use of information on acute viremia (early viral load) when this is available. We found that calculating the duration of an acute infection based on the acute viral load was more accurate than when the viral load result was treated as a qualitative (“positive”) result alongside other results to determine a “stage”. Importantly, this ability to back-calculate infection time is only applicable during the “ramp-up” phase of initial viremia—thus, an acute viral load should only be used to estimate infection time if it appears to be from the ramp-up period, e.g. it is known to be from a day when there was a negative antibody test. “Step 1” of the 2-step method is therefore to determine whether the acute viral load-based estimate can be used. If not, infection duration is estimated using the remaining complete testing history, considering all qualitative results, including qualitative interpretation of viral load results.

In addition to showing some advantages of the new method, we also independently validated the performance of previous staging systems. This exercise provides a basis for standardizing results across studies or programs that used older methods and those that will use the new method. It also provides reassurance that many acute infection studies that used Fiebig or 4th gen staging data may not need to be reanalyzed, as the accuracy advantage to the new method was relatively small (on the order of half a week). For some types of studies—such as acute HIV intervention studies, where timing in first 1–2 weeks of acute viremia may be critical—the error inherent in Fiebig and 4th gen systems may warrant focused re-analyses.

In other research settings (e.g., vaccine trials), or when making decisions about clinical acute infection treatment, the enhanced flexibility of assessing timing without an antibody-negative but viremic sample may be the more important feature of the new proposed system. In many of these situations, the interval defined by a seroconversion testing history may be many months or even years—or a last negative HIV test may not actually be documented. In these cases, it is possible that additional tests that measure the slow maturation of the HIV antibody response will provide more information. At present, these “tests for recent infection” (e.g., the LAg Avidity assay and others) are recommended only for use in population surveys²⁶ and not for use in estimating individual-level infection timing. If appropriate criteria could be developed to incorporate these data to improve individual timing estimates, this could further expand opportunities for improved clinical and public health management of newly diagnosed HIV infections.

It is important to note that some patient or viral characteristics (e.g. hepatitis C co-infection,^{27, 28} viral type,²⁹ use of HIV pre-exposure prophylaxis (PrEP),^{30, 31} or very early treatment during acute HIV infection³² can delay antibody seroconversion and/or viral ramp up in an

individual, leading to unexpected (and inaccurate) results from any of the methods described here. Further research is needed into the impact of these increasingly common factors on antibody and viral kinetics, particularly as they affect results of HIV diagnostic or incidence assays.

We have provided expanded guidance as well as updated diagnostic delay tables and tools for computing EDDIs and plausible intervals online, at <https://tools.incidence-estimation.org/idt/>. The tool facilitates calculation of an EDDI and plausible interval for almost any individual with at least one negative and one positive HIV test result, with the interval bound by the EP-DDI and LP-DDI. For many applications, the EP-DDI and LP-DDI may be more useful than the EDDI itself: examples might include determining subject eligibility for an acute intervention, talking to a patient about partners who may have been exposed during the plausible interval, or selecting specimens for inclusion in a research study. Researchers with access to cohorts with both multiple HIV test results and independent estimates of a plausible date of infection may pursue validation of our methods compared to their gold standard, understanding that there are known differences between estimated DDI and date of infection. Note that names for assays are sometimes regional; readers unsure about assays with the same or similar name are advised to refer to original source publications for clarification of assays and estimated diagnostic delays, available within the tool. More details about the analytical framework underlying this tool are available in Grebe *et al.* (forthcoming). This publicly available tool should be useful to researchers, clinicians or public health program staff wishing to use the methods described here to explore how to estimate DDI information from seroconversion testing histories; and will be updated as methods are further developed based on additional research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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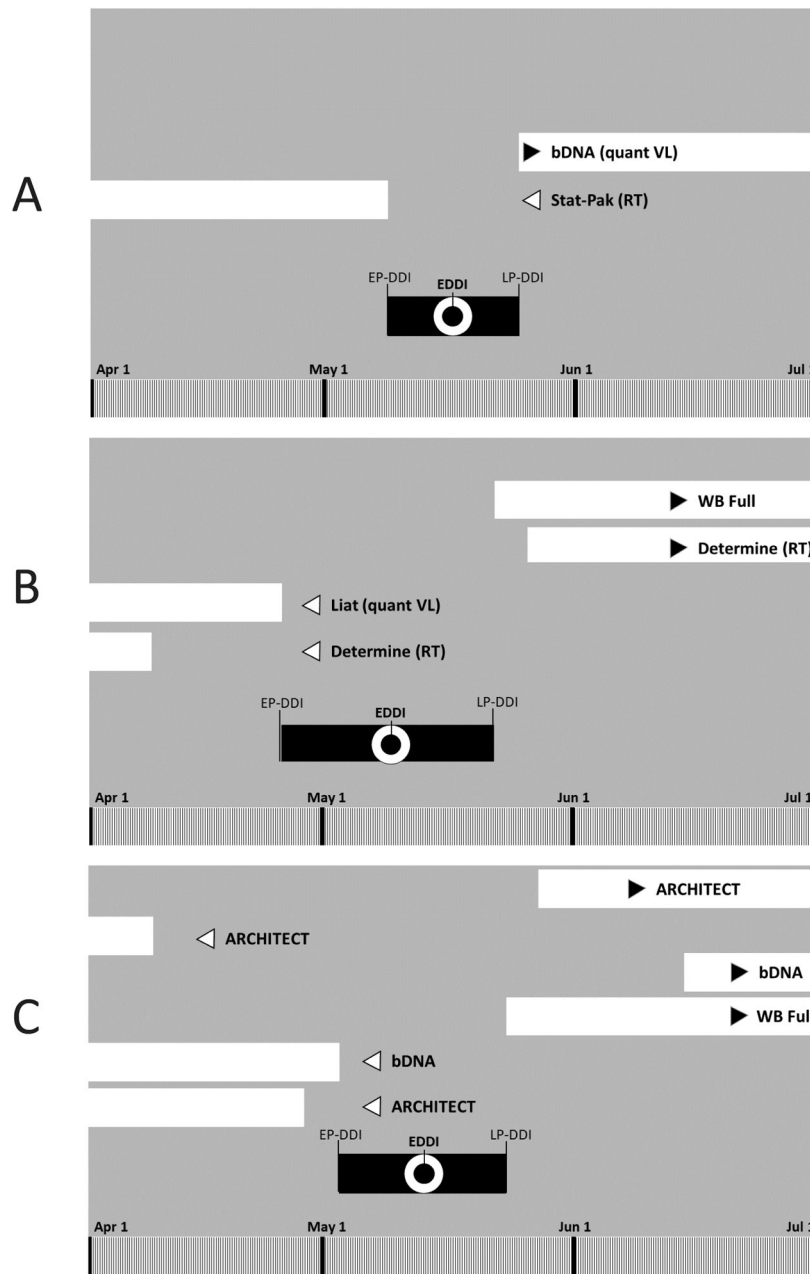


Figure 1. The testing history method.

The panels illustrate how an estimated date of detectable infection (EDDI), and a “plausible interval” for this estimate, can be inferred from HIV testing histories that include the dates and qualitative results on each HIV assay used. Testing history timelines are shown for three representative HIV seroconverters, with hypothetical dates of tests inputted and date of detectable infection (DDI) outputs shown along the x-axis as calendar days. Triangles represent HIV tests performed (solid=positive; empty=negative). The “diagnostic delay” from first detectable infection to initial positivity has been determined experimentally for most assays (see Table S1). For any result, the diagnostic delay for the assay used can be subtracted from the test date, to give an earlier date which is interpreted differently

depending on whether the result is now negative or positive. If the result is negative, we infer that the patient's actual DDI probably occurred sometime after that earlier date (else we would expect the assay result to now already be positive). If the result is positive, we infer that the DDI had probably already occurred by the earlier date (else, the result would still likely be negative). In the figures, white bars show ranges for the EDDI that are inconsistent with the particular result. Taken across all results in the history, these define the "earliest-and latest-plausible dates of detectable infection" (EP-DDI and LP-DDI). The "plausible interval" for the EDDI is bounded by these dates as shown by the black bar. The best estimate of the EDDI from the qualitative HIV testing history is then the midpoint of this plausible interval (shown by the circle). The method applies to discrepant qualitative HIV test results obtained on the same day (e.g., "antibody negative/RNA positive" (**Panel A**), but also applies when discrepant HIV results were obtained on two (**Panel B**) or multiple (**Panel C**) encounters: information from multiple test encounters may narrow the plausible interval and hence improve precision of the EDDI. Additional abbreviations: quant = quantitative; VL = viral load; RT = rapid test; WB = Western blot; Full = fully reactive assay.

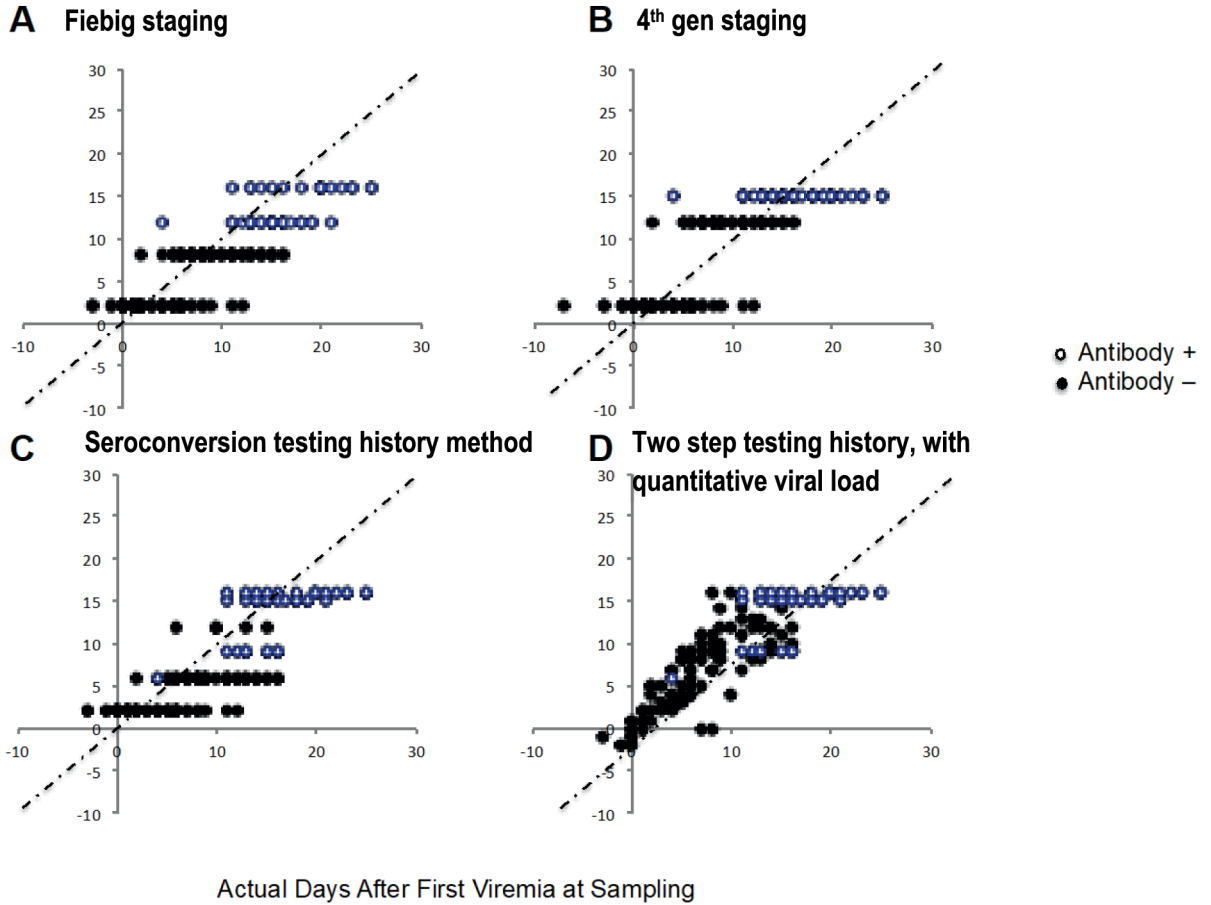


Figure 2. Estimates obtained using alternative staging methods, plotted versus time since infection (days since DDI₁₀₀).

In each figure, infection times predicted by each method (y axis) are compared with observed times (x axis) for 206 specimens from 31 seroconverters frequently donating blood plasma (all available data from all panels, including those used in model development and those used in model validation). Infection times are given as days since first viremia detected at the 100 RNA-copy/mL threshold (DDI₁₀₀); timing of first viremia was estimated within <3.5 days. Black circles represent specimens that were negative on the Western blot, while specimens with indeterminate Western blots are shown with white (unfilled) circles. Plots (A) and (B) show the distributions of infection times predicted by Fiebig² and 4th gen³ staging methods, respectively. Plot (C) shows the distribution of times for the seroconversion testing history method only, “step 2” of the 2-step method described in Figure 1, using the diagnostic delays in Table S1 for specimens with discrepant results occurring on the same day. Plot (D) shows the distribution of times for the 2-step method. While the testing history method can take all results into account, the plot again shows results limited to tests performed on the same day. In all four plots results were used from five assay types: viral load, p24 antigen ELISA, IgG-only, IgM/IgG antibody immunoassays and Western blot. In Plot (D), for antibody positive specimens only the qualitative results on the five assay types were used; for antibody negative specimens the method also used quantitative viral load information.

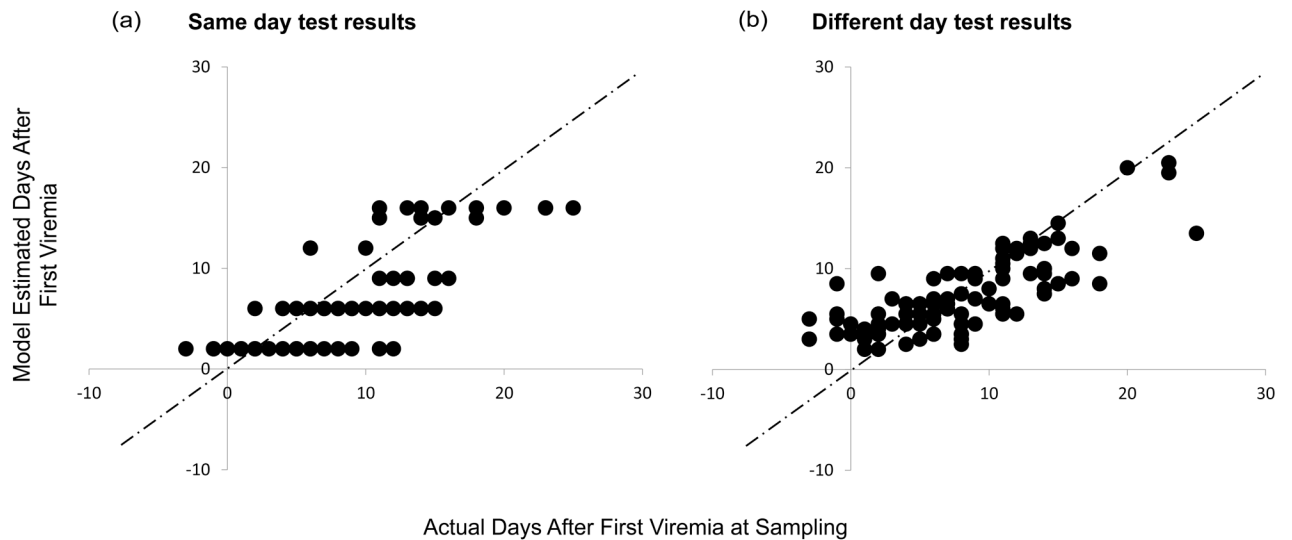


Figure 3. Estimates of infection duration obtained using discrepant, same-day qualitative test results (A) or using test results obtained from the same subjects on two different days (B). Estimates of time since first viremia detectable at 100 RNA copies/mL (DDI_{100}) are shown on the y axis for alternative scenarios of all panel observations using the testing history method, versus observed times on the x axis. In the first same-day scenario (**Panel A**), time was calculated based on results occurring on that day. In the serial testing scenario (**Panel B**), the data from that date were ignored and instead, test results from the visit prior to and after that visit were used to generate the DDI_{100} . In both scenarios results were used from five assay types: viral load, p24 antigen ELISA, IgM-sensitive and recombinant IgG-sensitive antibody immunoassays, and Western blot, and analysis was limited to $n=104$ specimens from $N=30$ individuals with results for all 5 assay types.

Performance of previously published Fiebig and 4th gen staging systems

Table 1.

Model and stage	Specimens	Predicted and observed times after first viremia (DDI ₁₀₀) for each stage			RMS difference (predicted-observed)
		Predicted time	Distribution of actual times observed	Error in model estimates	
	N/n	Median	[25 th ,75 th] IQR		
Fiebig (after Fiebig, et al., 2003)²					
Stage 1 RNA+ p24- 3G-	30/62	9 days	[8,12] days	3.5 days	
Stage 2 RNA+ p24+ 3G-	29/55	15	[14,19]	3.1	
Stage 3 RNA+ p24+ 3G+ 2G/WB-	15/27	19	[20,23]	4.5	
Stage 4 RNA+ WB indeterminate	10/18	23	[22,30]	6.8	
Stage 5 RNA+ WB+ (p31-)	8/54	61	[30,41]	26.2	
Stage 1-2* RNA+ 3G-	31/117	12	[9,16]	7.7	
Stage 1-3* RNA + Ab rapid test-	31/144	13	[10,20]	7.8	
Stage 1-4* RNA + unspecified Ab-	31/162	17	[11,21]	10.5	
4th Gen (after Ananworanich, et al. 2013)¹					
Stage 1 RNA+ 4G- 3G-	30/64	9	[8,12]	3.4	
Stage 2 RNA+ 4G+ 3G-	28/53	19	[14,19]	4.0	
Stage 3 RNA+ 4G+ 3G+ WB indeterminate	18/45	22	[21,27]	5.4	

Abbreviations: 3G = 3rd Generation (IgM-sensitive antibody assays); 4G = 4th Generation (p24 antigen/antibody assays); RMS = root mean square; IQR = interquartile range; DDI₁₀₀ = days since detectable infection on a 100-copy viral load; N = unique individuals; n = specimens from distinct timepoints

* Because it is often impossible to determine the precise Fiebig stage, examples are also given for the infection times seen for combinations of stages which can sometimes be inferred from 'incomplete' staging information

Table 2.

Performance of newer proposed prediction methods, as compared to complete Fiebig staging as a benchmark method

Method compared to Fiebig (reference standard)	Specimens	New method performance		Fiebig performance (benchmark)		Method comparison
	N ⁵ /n ⁶	ICC ¹	RMS Difference ²	ICC	RMS Difference ²	P value ³
4 th gen staging system	31/162	0.779	4.2	0.756	4.2	0.96
Quantitative RNA viral load model (Ab⁴ - specimens only)						
IgM-sensitive Ab test (-) specimens only	14/41	0.899	2.2	0.591	3.5	< 0.001
Any Ab test (-) specimens	14/51	0.877	2.6	0.754	3.4	0.21
Testing history only (quantitative viral load not entered)	31/161	0.744	4.4	0.758	4.2	0.09
Testing history, including quantitative RNA viral load	31/157	0.834	3.5 days	0.759	4.1 days	0.002

¹ ICC = intraclass correlation of model predictions with observed infection duration

² RMS = root mean square – difference between predicted and observed data was calculated as a measure of estimation error

³ P values shown indicate whether the estimation error (RMS difference) is statistically different with the newer model, as compared to with the benchmark model

⁴ Ab = Antibody

⁵ N = unique individuals

⁶ n = specimens from distinct timepoints

Sensitivity of “testing history” method estimates to the type of testing history information available

Table 3.

Staging scenarios compared	Information used by the “testing history method”		Specimens	Model performance: predicted vs. observed infection duration	
	Results included at each observation ¹	Observations included		ICC	RMS difference ²
“Fiebig-like”: same-day tests, vs. results from different days	RNA (qualitative), p24, 3G, 2G, WB RNA (qualitative), p24, 3G, 2G, WB	Same day Two days (two bleeds apart)	30/104 30/104	0.698 0.715	4.1 3.7*
Newer Assays	RNA (qualitative), 3G, WB RNA (qualitative), 4G, Geenius	Same day Same day	14/64 14/64	0.739 0.698	4.3 5.3*

* statistically significant difference in prediction accuracy between model scenarios at a level of p<0.05.

Abbreviations: ICC = intraclass correlation; RMS = root mean square; 3G = 3rd Generation (IgM-sensitive antibody assays); 2G = 2nd Generation (Recombinant IgG-sensitive antibody assays); 4G = 4th Generation (p24 antigen/antibody assays); WB = Western blot; N = unique individuals; n = specimens from different timepoints

¹ For these analyses the testing history was assumed not to include the quantitative viral load.

² RMS = root mean square – difference between predicted and observed data was calculated as a measure of estimation error