Supplementary Material

Precision detection of recent HIV infections using high-throughput genomic incidence assay

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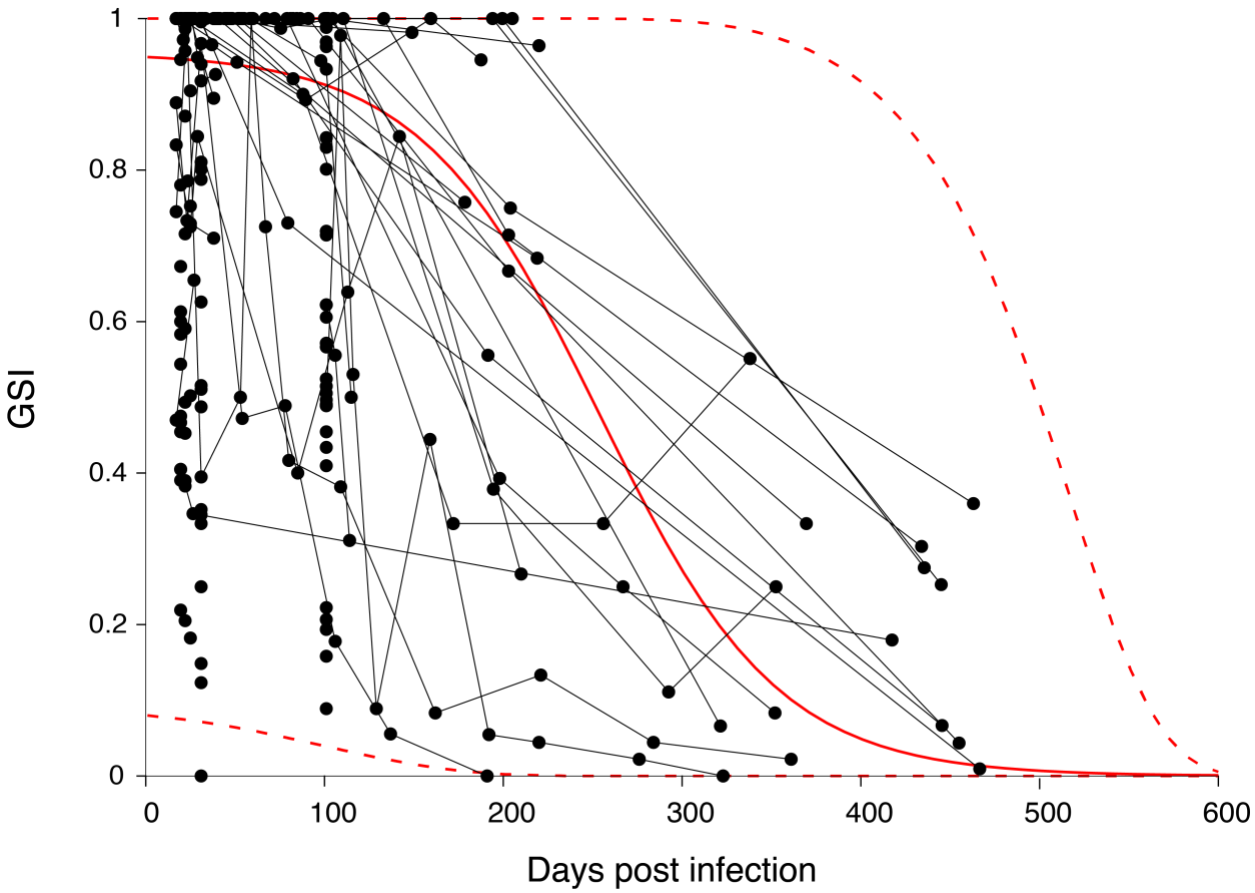
Infection time estimates by HIV RNA test dates and seroconversion dates

The first sample of SC4 was collected on December 8th, 2009. This individual's HIV RNA last negative and first positive dates were March 14th, 2008, and November 17th, 2008, respectively. Therefore, we estimated that SC4's first sample was collected between 21 and 269 days after infection (Table 2). At the time of the first RNA positive date, this study participant was seronegative and thus at Fiebig stage I or II. By adding the estimated duration of Fiebig stage I or II (19.5 [13-34] days) to the elapsed time between the RNA first positive date and the date of specimen collection (21 days) [1, 2], we obtained an estimated infection duration of 40.5 [34 - 55] days for the first sample. This Fiebig staging estimate was within the interval obtained from HIV RNA test dates. Throughout the course of more than a year, eight additional samples were collected from this study participant (Table 2). The time since infection for each subsequent sample was estimated by adding the sample collection interval to the first sample's estimate.

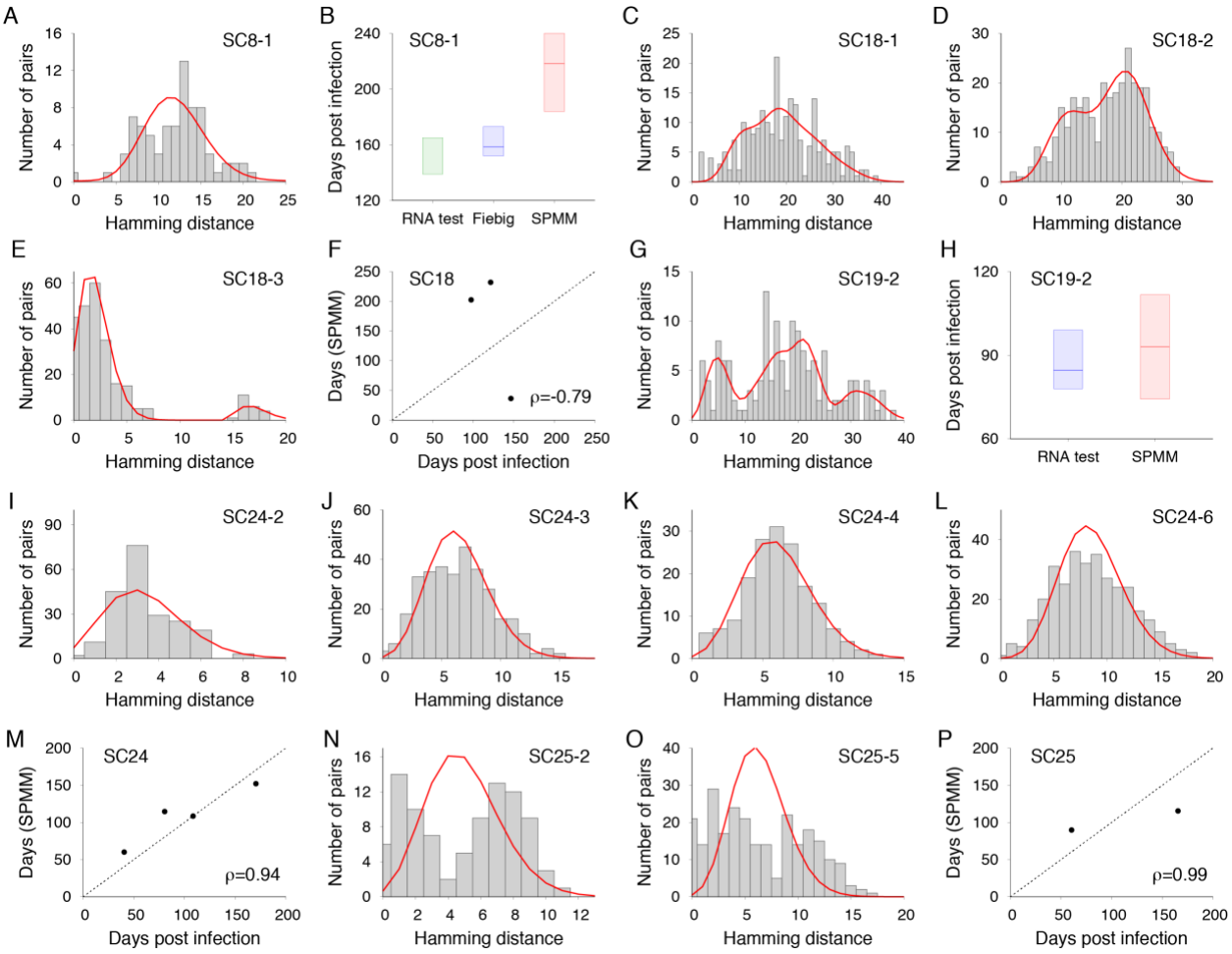
Other study participants, SC8, SC15, SC18, SC19, SC20, SC22, SC23, SC24, and SC25 were also seronegative when the first sample was collected and the time since infection was estimated using the Fiebig estimate (Table 1). The first sample collected from study participant SC5 was seropositive, but it was estimated that this sample was taken within 77 days post infection based on the individual's HIV RNA negative test date. A middle time point of 38.5 days was used as the estimate for the time of infection. The HIV RNA negative and positive test dates for study participant SC21 indicated that the first seropositive sample was collected within 609 days since infection (Table 1). Instead of the middle point estimate, the shifted Poisson mixture model [3] was used to estimate time since infection, as detailed below.

Sources for publicly available incident and chronic specimens

We collected publicly available HIV complete envelope gene sequences as previously described [4, 5]. A total of 417 incident specimens were used to estimate GSI distribution over time, which comprised of 252 incident specimens at Fiebig stages I, II, III, IV, and V [2, 6-18] and 165 incident longitudinal specimens obtained from 43 individuals [2, 6, 7, 9, 11, 16, 19-21]. An additional 107 publicly available incident specimens were used to measure the detection accuracy of recent infections [22]. A total of 162 publicly available chronic specimens with an infection time longer than one year were analyzed to determine the false recency rate (FRR) [2, 17, 19, 23-42].



Supplementary Figure 1. Modeling biomarker dynamics. A total of 417 publicly available incident samples with infection times estimated by Fiebig staging and sample collection intervals [16, 17]. Data points with line segments represent serial samples collected from individuals. The maximum likelihood estimates of the model parameters were as follows: c = 0.95 [0.94 – 0.96], M = 253.8 [220.2 – 292.2], S = 50.1 [37.9 – 67.9], and V = 1.00 [0.94 – 1.05]. To obtain the 95% confidence interval (CI) for each parameter, we resampled the 417 incident specimens with replacement 1,000 times. The fitted mean for GSI dynamics was presented as a red solid line and 99% prediction intervals were presented as red dotted lines over days post infection.



Supplementary Figure 2. Time since infection estimated by shifted Poisson mixture model (SPMM). A. The fit of SPMM (red line) to the Hamming distance distribution of SC8-1's 13 envelope gene sequences (grey boxes). **B.** Time since infection estimated by SPMM, 218.2 [183.9 – 252.5], was greater than HIV RNA test date estimate of [139 – 165] and Fiebig staging estimate of 158.5 [152 – 173] days. **C.** The fit of SPMM to the Hamming distance distribution of SC18-1. **D.** The fit of SPMM to the Hamming distance distribution of SC18-2. **E.** The fit of SPMM to the Hamming distance distribution of SC18-3. **F.** The model estimates were not consistent with HIV RNA test date estimates and Fiebig staging (Pearson correlation coefficient $\rho = -0.79$). **G.** The fit of SPMM to the Hamming distance distribution of SC19-2. **H.** The model estimate agreed with the infection time range determined by dates of the last negative and first positive HIV RNA tests. **I.** The fit of SPMM to the Hamming distance distribution of SC24-2. **J.** The fit of SPMM to the Hamming distance distribution of SC24-3. **K.** The fit of SPMM to the Hamming distance distribution of SC24-4. **L.** The fit of SPMM to the Hamming distance distribution of SC24-6. **M.** SPMM's infection time estimates were consistent with Fiebig estimates for the SC24's four samples ($\rho=0.94$). **N.** The fit of SPMM to the Hamming distance distribution of SC25-2. **O.** The fit of SPMM to the Hamming distance distribution of SC25-5. **P.** The model estimates were consistent with Fiebig estimates ($\rho=0.99$).

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