

New Testing Strategy to Detect Early HIV-1 Infection for Use in Incidence Estimates and for Clinical and Prevention Purposes

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Context.—Differentiating individuals with early human immunodeficiency virus 1 (HIV-1) infection from those infected for longer periods is difficult but important for estimating HIV incidence and for purposes of clinical care and prevention.

Objective.—To develop and validate a serologic testing algorithm in which HIV-1–positive persons with reactive test results on a sensitive HIV-1 enzyme immunoassay (EIA) but nonreactive results on a less sensitive (LS) EIA are identified as having early infection.

Design.—Diagnostic test and testing strategy development, validation, and application. Specimens were tested with both a sensitive HIV-1 EIA (3A11 assay) and a less sensitive modification of the same EIA (3A11-LS assay).

Settings and Participants.—For assay development: 104 persons seroconverting to HIV-1 comprising 38 plasma donors, 18 patients of a sexually transmitted disease clinic in Trinidad, and 48 participants in the San Francisco Men's Health Study (SFMHS); 268 men without the acquired immunodeficiency syndrome (AIDS) in the SFMHS who had been infected for at least 2.5 years; and 207 persons with clinical AIDS; for testing strategy validation: 488 men in the SFMHS from 1985 through 1990 and 1 275 449 repeat blood donors at 3 American Red Cross blood centers from 1993 through 1995; and for HIV-1 incidence estimates: 2 717 910 first-time blood donors. We retrospectively identified persons eligible for a study of early infection.

Main Outcome Measure.—Ability to identify early HIV infection.

Results.—Estimated mean time to being 3A11 reactive/3A11-LS nonreactive was 129 days (95% confidence interval [CI], 109-149 days). Our testing strategy accurately diagnosed 95% of persons with early infection; however, 0.4% (1/268) of men with established infection and 2% (5/207) of persons with late-stage AIDS were misdiagnosed as having early HIV-1 infection. Average yearly incidence estimates in SFMHS subjects were 1.5% per year vs observed average incidence of 1.4 per 100 person-years. Incidence in repeat blood donors using the sensitive/less sensitive assay testing strategy was 2.95 per 100 000 per year (95% CI, 1.14-6.53/100 000) vs observed incidence of 2.60 per 100 000 person-years (95% CI, 1.49-4.21/100 000). Overall incidence in first-time blood donors was 7.18 per 100 000 per year (95% CI, 4.51-11.20/100 000) and did not change statistically significantly between 1993 and 1996. Use of the sensitive/less sensitive testing strategy alone would have identified all 17 persons with antibodies to HIV-1 eligible for a study of early HIV-1 infection and would have increased enrollment.

Conclusions.—The sensitive/less sensitive testing strategy provides accurate diagnosis of early HIV-1 infection, provides accurate estimates of HIV-1 incidence, can facilitate clinical studies of early HIV-1 infection, and provides information on HIV-1 infection duration for care planning.

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MONITORING and controlling the spread of human immunodeficiency virus 1 (HIV-1) worldwide^{1,2} would greatly benefit from a simple, practical method of identifying recently infected persons. Accurate and rapid diagnosis of recent infection also has clinical implications.

Recent HIV-1 infection comprises the preseroconversion period from exposure to detection of antibodies by enzyme immunoassay (EIA) and Western blot and the early postseroconversion period. The antibody-negative period is referred to as acute or primary HIV-1 infection, diagnosed by p24 antigen^{3,4} or viral RNA via polymerase chain reaction^{5,6} and during which symptoms of the

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During initial development of the 3A11-LS assay, Dr Stramer was employed by Abbott Laboratories. After drafting of this article, Dr Satten served as a consultant for Abbott Laboratories.

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acute retroviral syndrome occur.^{4,6,7} The period immediately after seroconversion, which we refer to as early HIV-1 infection, has been difficult to diagnose with a single blood specimen.

Serologic monitoring of the HIV-1 epidemic has been generally limited to monitoring seroprevalence, the proportion of persons with HIV-1 antibodies, comprising both those with early infection and those with chronic infection.⁸ The best data for understanding recent changes in transmission are measurements of the number of new infections in a defined time period (incidence) that have been primarily provided by longitudinal cohort studies,^{9,10} longitudinal studies of persons at risk for infection who seek repeat HIV testing,¹¹ and longitudinal unlinked serosurveys.¹² These studies are technically difficult and expensive, require long follow-up, and may be biased.¹³

The detection of HIV-1 p24 antigen in the preseroconversion period has been proposed for identifying persons with acute infection for estimating incidence.¹³⁻¹⁵ However, because the duration of antigenemia before antibody detection is brief (average, 14 [G.A.S., unpublished data, 1998]-22 days^{14,16}), that approach is limited to situations in which a large number of people can be tested or in which the population incidence is high (eg, more than 5% per year). Using this approach, incidence surveys that oversample persons likely to have symptoms related to the acute retroviral syndrome will overestimate incidence in the population from which the sample is selected.

To identify a person in the period of early infection (when the antibody titer is increasing but before peak and persistently high antibody response¹⁷⁻¹⁹), we propose a sensitive/less sensitive assay testing algorithm in which a blood specimen from a person with early infection is reactive on an EIA sensitive to antibodies but nonreactive on a less sensitive EIA. This testing strategy estimates incidence by equating incidence with prevalence of persons with early infection divided by the time between seroconversion on the 2 tests.²⁰

METHODS

Assays

For the sensitive assay, we used a Food and Drug Administration (FDA)-licensed EIA (3A11, Abbott Laboratories, Abbott Park, Ill) as recommended by the package insert.²¹ Specimens with reactive 3A11 test results were confirmed to have HIV-1 antibodies by Western blot.²² For the less sensitive EIA (called 3A11-LS in this article), we modified 3 elements of the 3A11 test procedure: sample dilution, sample incubation time,

Table 1.—Sources of Serial Serum or Plasma Specimens From Persons Seroconverting to HIV*

Source	No. of Persons	No. of Specimens	Time Between Specimens, Median (Range), d	Duration of Follow-up, Median (Range), d
Plasma donors†	38	314	5 (1-111)	50 (18-431)
Trinidad STD clinic	18	149	16 (5-686)	342 (88-767)
SFMHS	48	227	210 (160-1091)	2221 (238-4012)

*Each person has at least one 3A11 nonreactive and one 3A11 reactive specimen. HIV indicates human immunodeficiency virus; STD, sexually transmitted disease; and SFMHS, San Francisco Men's Health Study.

†Obtained from a variety of commercial sources.

and conjugate incubation time. For the less sensitive assay, sample optical density (OD) value was standardized as follows: (sample OD value – negative control OD value)/positive control OD value. To further reduce sensitivity, we varied the cutoff OD above which a specimen was considered to have a reactive result.

Specimens for Assay and Testing Strategy Development

To identify optimal conditions for the 3A11-LS assay and determine duration of time between seroconversion on 3A11 and 3A11-LS assays, we obtained 690 serial plasma or serum specimens from 3 sources from 104 persons seroconverting to HIV-1 (Table 1).²³⁻²⁷ All specimens from plasma donors and the San Francisco Men's Health Study (SFMHS) were tested with the 3A11 assay and Western blot. All specimens from Trinidad had been screened by Western blot, and we confirmed the Western blot-positive specimens to be reactive by 3A11 assay.

To assess whether the 3A11-LS test remains reactive throughout infection, we obtained specimens from 268 men without the acquired immunodeficiency syndrome (AIDS) infected for at least 2.5 years and seen between July and December 1986 in the SFMHS, 49 persons (48 men, 1 woman) with clinical AIDS from Boston Biomedica, Inc, and the last available specimen from all 158 men with an AIDS diagnosis in the SFMHS. The CD4 cell counts from the same day as the serologic specimens from seroconverters and from persons with AIDS were assessed using the Wilcoxon rank sum test²⁸ to assess whether the CD4 cell count could be used to differentiate between these 2 groups in persons with 3A11 reactive/3A11-LS nonreactive results.

Development of Optimal 3A11-LS Test Conditions

All specimens from plasma donors and the SFMHS were tested using 3 combinations of modifications of the 3A11 assay that made it less sensitive (data not shown). We chose a 1:20 000 sample dilution, 30-minute sample incubation time, and 30-minute conjugate incubation time for the conditions for the less sensitive assay because, when using them, the 3A11-LS is easy to perform on large numbers of samples, has low sensitivity, and

achieves reproducibility similar to the 3A11 assay.²¹ Interassay reproducibility of the 3A11-LS assay conditions was assessed by testing samples in 2 laboratories and using 3A11 kits from the same master lot number. Assay-positive and assay-negative controls were tested 10 times in 1 run. Percent coefficient of variation (%CV) for positive controls was 7.8 (mean $\pm$ SD OD, 0.332 $\pm$ 0.026) and for the negative controls was 18.2 (mean $\pm$ SD OD, 0.022 $\pm$ 0.004). Regarding intra-assay reproducibility, results were analyzed from triplicate tests of internal assay positive controls from 9 assay runs from which mean %CV was 11.6 (mean $\pm$ SD OD, 0.396 $\pm$ 0.046).

Once we chose optimal 3A11-LS test conditions, all Trinidad specimens were tested with the 3A11-LS assay. Specimens from any of the 3 sources with results unexpectedly high or low for their sequence in a subject's sample series, and, when available, specimens with OD values between 50% and 150% of the internal assay positive control were retested in triplicate for verification. For specimens tested in triplicate, we compared mean OD of the 3 samples with the cutoff to assess sample reactivity. In calculating the mean of specimens tested in triplicate, OD values greater than 50% less or 100% more than the closest OD value were excluded. Using OD cutoffs of 0.50, 0.75, or 1.00, 76% to 82% of 125 specimens tested in triplicate had the same reactive or nonreactive results as those of the original single 3A11-LS test, similar to those for comparing initial samples with repeatedly reactive samples on the 3A11 assay.²¹

Model Estimating Time Between Seroconversion on the 2 Assays

We estimated distribution and mean time between seroconversion on the 3A11 assay and the 3A11-LS assay using a mathematical model for specimens from all 3 sources with a variety of cutoffs. To estimate time between seroconversion on the 2 assays, we assumed a progressive increase in antibody during early infection, producing for each subject a well-defined time on each assay before which results would be nonreactive and after which results would be reactive; seroconversion time on the 3A11 assay was uniformly distributed between time of the last 3A11 nonreactive specimen and the

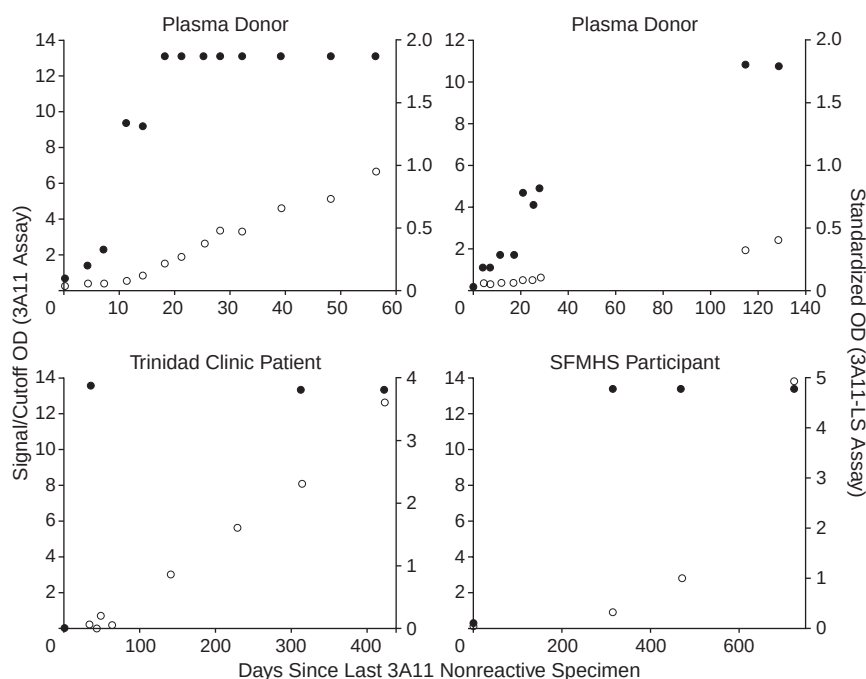


Figure 1.—3A11 (closed circle) and 3A11-LS (open circle) optical density (OD) test results from serial blood specimens from 4 illustrative individuals. The origin of the x axis is the day of the last 3A11 nonreactive specimen. Note that scales for the x and y axes vary among the figures and that different scales are used for the 3A11 and 3A11-LS results.

time of the first 3A11 reactive specimen; 3A11-LS assay seroconversion occurred no earlier than 3A11 assay seroconversion; and time difference between seroconversion on the 3A11 and 3A11-LS assays was independent of seroconversion time on the 3A11 assay. We modeled time between seroconversions using a discrete distribution that assigned a probability to each day from 0 to 3000 days, estimated by maximum likelihood based on observed data on times of last nonreactive and first reactive results for 3A11 and 3A11-LS assays, using an EM algorithm approach.²⁹ A smoothing step was added to the algorithm³⁰ to speed convergence and produce smooth curves; a kernel smoother with a triangular kernel was used with bandwidth (h) of 20 days. Mean times between 3A11 and 3A11-LS seroconversion were largely invariant for the range of days for smoothing bandwidths we considered ($0 \leq h \leq 100$). Confidence intervals (CIs) for mean time between seroconversions were obtained using the bootstrap percentile method.³¹ Day of 3A11 assay seroconversion was estimated from the model conditional on observed times of last nonreactive and first reactive results for 3A11 and 3A11-LS assays and using estimated distribution of times between seroconversions. To assess ability of the testing strategy to accurately classify specimens obtained within 129 days of estimated day of 3A11 seroconversion and to correct for mul-

multiple specimens provided by subjects, we calculated the average proportion of each person's specimens with 3A11 reactive/3A11-LS nonreactive results obtained in that period.

Sensitive/Less Sensitive Testing Strategy for Estimating HIV-1 Incidence

We estimated incidence with the sensitive/less sensitive testing strategy with the following formula: $I_{dt} = (n_{dt}/N) (365/T) (100)$, where I_{dt} is incidence (percent per year); n_{dt} is number of persons who were 3A11 reactive/3A11-LS nonreactive; N is number of persons who were HIV-1 negative plus number who were 3A11 reactive/3A11-LS nonreactive; and T is estimated mean number of days between seroconversion on 3A11 and 3A11-LS tests (Appendix 1).

To determine whether the sensitive/less sensitive testing strategy gives accurate incidence estimates, we compared our estimates with observed incidence in 2 groups with different incidences. Observed incidence was calculated as number of persons seroconverting between sequential follow-up visits divided by person-years of follow-up. In the first validation, we examined twelve 6-month follow-up periods of the SFMHS. To provide independent verification for this comparison, we used an estimate of mean time between seroconversion on the 3A11 and 3A11-LS assays using data only

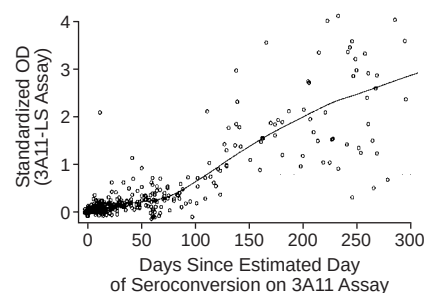


Figure 2.—Plot of standardized 3A11-LS optical density (OD) values of specimens obtained since estimated day of seroconversion on 3A11 assay. All specimens were aligned at origin of x axis by imputing a day of seroconversion on 3A11 assay (see "Methods"). The solid line is a lowess³⁴ smooth of the OD values. The dashed line represents an OD cutoff of 0.75 for the 3A11-LS assay.

from plasma donor and Trinidad specimens. In the second validation, in a population with low incidence, we tested the HIV antibody-positive blood donations from persons donating more than once from January 1993 through December 1995 in 3 American Red Cross collection regions (Appendix 2) that participate in the Retrovirus Epidemiology Donor Study (REDS).³² Donations that tested HIV-1 positive with only 2 bands on Western blot were tested by RNA polymerase chain reaction as recommended to rule out false-positive results in donors.³³

Applications of the Sensitive/Less Sensitive Testing Strategy

To better understand current HIV-1 transmission on a national level, we estimated incidence and examined trends in proportion with early infection in first-time donors at 32 American Red Cross collection regions (about 70% of annual Red Cross donations) from May 1993 through December 1996 using χ^2 test for linear trend.²⁸

To examine utility of the sensitive/less sensitive testing strategy in clinical research, we retrospectively tested specimens of potential enrollees in the Options Project, a research study of treatment, epidemiology, and pathogenesis of primary HIV infection in San Francisco. Criteria for study enrollment were having negative or indeterminate Western blot and positive HIV-1 RNA test results, or having a documented negative antibody result less than 6 months before a positive antibody result at enrollment.

RESULTS

Results from 3A11 and 3A11-LS testing of serial specimens from 4 illustrative individuals are shown in Figure 1. Signal/cutoff OD values on the standard 3A11 assay increase rapidly over several weeks while standardized OD val-

ues on the 3A11-LS assay increase more gradually. Figure 2 shows standardized OD values from the 3A11-LS assay for 372 specimens from 104 persons seroconverting to HIV-1 and shows the increase in 3A11-LS reactivity with increasing time after the estimated day of 3A11 seroconversion.

Figure 3, left, shows the estimated distribution of and Table 2 the mean time between seroconversion on the 3A11 and 3A11-LS assays from our model. Increasing the cutoff on the 3A11-LS assay increases mean time between seroconversion on the assays. We selected an

OD cutoff value of 0.75 for the 3A11-LS assay for our analyses because patterns of times between specimen collection and length of follow-up for each of the 3 data sources resulted in this cutoff's producing the most reliable estimate of distribution of times between seroconversion on the 2 tests. Mean time between seroconversion on the assays was 112 days for plasma donor samples and 127 days for samples from Trinidad. Because intertest intervals were so long for the SFMHS, the (unsmoothed) maximum likelihood estimate (MLE) of distribution of times between seroconversions

using only SFMHS data has a large region where the MLE form is not fully specified, although total mass amount assigned to the region is specified.³⁵ By assuming extreme possibilities (all mass at beginning or end of the undefined region), range of mean time between seroconversions was 71 to 143 days. For all data sources combined, this cutoff yields a 129-day (95% CI, 109-149 days) interval between seroconversion on the 2 assays. Figure 3, right, shows the average percentage of each person's specimens that were 3A11 reactive/3A11-LS nonreactive by days since estimated day of seroconversion on the 3A11 assay. Similarity in shape of Figure 3, left and right, suggests our mathematical model appropriately describes time between 3A11 and 3A11-LS seroconversions.

In our study population, 77 (98.7%) of 78 persons providing a specimen within 129 days of estimated day of 3A11 seroconversion had at least one 3A11-LS nonreactive specimen. Average proportion of each subject's specimens that were 3A11-LS nonreactive was 95.4%. Of the 303 specimens provided within 129 days of estimated day of 3A11 seroconversion, 296 (97.7%) were 3A11-LS nonreactive. Conversely, of 60 persons with specimens more than 129 days after estimated day of 3A11 seroconversion, only 3 (5%) had at least one 3A11-LS nonreactive specimen. Of the 60, average proportion of each subject's specimens that were 3A11-LS nonreactive was 1%. Of 154 total specimens provided at least 129 days after estimated 3A11 seroconversion date, 3 (1.9%) were 3A11-LS nonreactive. The 3A11-LS test became reactive for all 57 seroconverting persons followed up for at least 149 days after estimated seroconversion date. In addition, only 1 (0.4%) of 268 specimens obtained

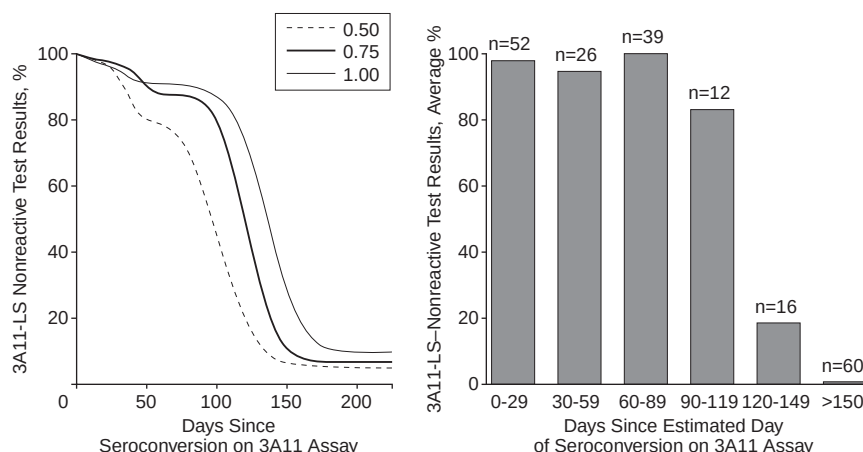


Figure 3.—Analyses of 3A11-LS results from specimens obtained after seroconversion on 3A11 assay. Left, From mathematical model (see “Methods”), distribution of time from seroconversion on 3A11 assay to seroconversion on 3A11-LS assay for 3 cutoff values (optical density [OD] = 0.50, 0.75, and 1.00) for the 3A11-LS assay. Right, Results of 3A11-LS testing (OD cutoff = 0.75) during selected periods after estimated day of seroconversion on 3A11 assay. Since persons provided multiple blood specimens in a period, height of each bar represents average percentage of each person's specimens with 3A11-LS nonreactive results obtained in the period. In most cases, multiple specimens provided by a subject during a period had the same test results; however, in the 30- to 59-day period, 1 person provided 3 3A11-LS nonreactive specimens followed by a 3A11-LS reactive specimen, and in the greater than 150-day period, 3 persons each provided a specimen with 3A11-LS nonreactive results followed by multiple blood specimens with 3A11-LS reactive results.

Table 2.—Effect of Varying OD Cutoff for 3A11-LS Assay on Sensitive/Less Sensitive Testing Strategy*

Cutoff OD for 3A11-LS Test	Time Between Seroconversion on the 3A11 and 3A11-LS Assays, Mean (95% CI), d	No. (%) of Specimens From Persons With Early Infection With 3A11 Reactive/3A11-LS Nonreactive Results†	Median CD4 Cell Count of Specimens From Early Infection With 3A11 Reactive/3A11-LS Nonreactive Results, ×10 ³ /L (No. of Specimens)‡	Persons With Established HIV Infection, but Not AIDS, Classified as Having Early Infection, % (n = 268)§	Persons With AIDS Classified as Having Early Infection, % (n = 207)§	Median CD4 Cell Count of Specimens From Persons With AIDS Testing 3A11 Reactive/3A11-LS Nonreactive, ×10 ³ /L (No. of Specimens)	Sample Size Needed If Incidence	
							=1% and 95% CI = 0.3-3.0	=5% and 95% CI = 2.5-10.0
0.50	102 (83-121)	290 (95)	0.454 (73)	0	1.4	0.027 (3)	3600	2000
0.75	129 (109-149)	303 (98)	0.446 (90)	0.4	2.4	0.027 (5)	3000	1400
1.00	164 (134-199)	317 (96)	0.454 (105)	0.7	2.9	0.044 (6)	2300	1400
1.25	184 (152-238)	326 (94)	0.466 (111)	1.1	3.4	0.075 (8)	¶	¶
1.50	202 (170-252)	330 (95)	0.491 (122)	1.1	5.3	0.048 (13)	¶	¶
2.00	252 (200-321)	352 (95)	0.495 (139)	1.9	10.1	0.038 (23)	¶	¶

*Varying the cutoff on the 3A11-LS assay affects the time between seroconversion on the 3A11 and 3A11-LS assays, the proportion of persons with later-stage disease misclassified as having early infection, and the sample size needed for cross-sectional incidence studies. OD indicates optical density; LS, less sensitive; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; and CI, confidence interval.

†Number of 3A11-LS nonreactive specimens obtained from persons within the cutoff-specific estimated mean number of days after seroconversion on the 3A11 assay.

‡CD4 cell counts of 3A11 reactive/3A11-LS nonreactive specimens from persons seroconverting to HIV. CD4 cell counts were available only from specimens from Trinidad and the San Francisco Men's Health Study.

§Proportion with 3A11 reactive/3A11-LS nonreactive test results.

||Means and 95% CIs for cutoffs greater than 1.00 are conditional on seroconversion. For cutoffs of 1.25, 1.50, and 2.00, we estimate that approximately 2% of persons with HIV infection may never have a reactive 3A11-LS test result.

¶Cutoffs greater than 1.00 for the 3A11-LS assay produce greater uncertainty in the estimate of the time between seroconversion on the 2 tests than do lower cutoffs and may not provide incidence estimates as accurately as with lower cutoffs.

Table 3.—Comparison of HIV Incidence in the San Francisco Men's Health Study, Estimated by the Sensitive/Less Sensitive Testing Strategy, With Observed Estimates*

Period in Which Seroconversion on 3A11 Assay Occurred	Observed HIV Incidence		Estimation by Sensitive/Less Sensitive Testing Strategy†		
	No. Observed to Seroconvert	Incidence per 100 Person-Years (95% CI)	No. Identified as Having Early Infection‡	No. at Risk for HIV Infection During Period	Incidence, % per Year (95% CI)
1/85-6/85	10	3.6 (1.7-6.6)	5	488	3.1 (0.8-9.2)
7/85-12/85	5	2.4 (0.8-5.6)	2	408	1.5 (0.1-7.0)
1/86-6/86	5	2.4 (0.8-5.7)	1	393	0.8 (0.01-5.7)
7/86-12/86	5	2.5 (0.8-5.8)	3	398	2.3 (0.3-8.6)
1/87-6/87	3	1.2 (0.3-3.6)	1	405	0.8 (0.01-5.5)
7/87-12/87	1	0.5 (0.01-3.0)	1	393	0.8 (0.01-5.7)
1/88-6/88	2	1.0 (0.1-3.6)	2	393	1.6 (0.1-7.3)
7/88-12/88	2	1.0 (0.1-3.5)	2	396	1.6 (0.1-7.2)
1/89-6/89	2	0.9 (0.1-3.3)	2	408	1.5 (0.1-7.0)
7/89-12/89	1	0.5 (0.01-2.6)	1	391	0.8 (0.01-5.7)
1/90-6/90	1	0.5 (0.01-2.8)	1	391	0.8 (0.01-5.7)
7/90-12/90	1	0.6 (0.01-3.5)	0	266	0 (0.0-4.9)
Average§	3	1.4 (. . .)	2	394	1.5 (. . .)

*HIV indicates human immunodeficiency virus; CI, confidence interval; and LS, less sensitive.

†Calculations using the sensitive/less sensitive testing strategy used a mean time between seroconversion on the 3A11 and 3A11-LS assays of 119 days (95% CI, 104-131 days) based on data only from plasma donor and Trinidad specimens.

‡Early infection is defined as 3A11 reactive/3A11-LS nonreactive specimens with an optical density cutoff of 0.75 for the 3A11-LS test.

§Unweighted average of 6-month results.

Table 4.—Trends in HIV Incidence Among Persons Donating Blood for the First Time in 32 American Red Cross Collection Regions, May 1993 Through December 1996*

Year	No. HIV Positive†	No. HIV Positive/ and 3A11-LS Nonreactive	No. Tested‡	Incidence per 100 000 per Year (95% CI)
1993§	107	15	460 507	9.22 (3.95-19.28)
1994	177	24	763 141	8.90 (4.47-16.62)
1995	141	12	754 551	4.50 (1.76-10.02)
1996	122	18	739 711	6.89 (3.16-13.74)
1993§-1996	547	69	2 717 910	7.18 (4.51-11.20)

*HIV indicates human immunodeficiency virus; LS, less sensitive; CI, confidence interval; and EIA, enzyme immunoassay.

†Donations were screened with a combination HIV-1/HIV-2 EIA and those with repeatedly reactive EIA results were confirmed to have antibodies to HIV by Western blot. All HIV-positive donations that had only 2 bands on Western blot were tested with RNA polymerase chain reaction. Polymerase chain reaction-negative donations were excluded from the analysis.

‡Includes those with negative HIV test results and those with HIV positive/3A11-LS nonreactive test results.

§May through December 1993. All other years are January through December.

from men without AIDS in the SFMHS was 3A11-LS nonreactive (Table 2).

Of 207 specimens from persons with AIDS, 5 were 3A11 reactive/3A11-LS nonreactive (Table 2). Of these, 4 were from men from the SFMHS for whom earlier specimens were available. Blood specimens from 6 months before their 3A11 reactive/3A11-LS nonreactive tests were reactive on both assays. The CD4 cell counts were significantly higher in seroconverters who were 3A11 reactive/3A11-LS nonreactive (mean $\pm$ SD CD4 cell count, $0.554 \pm 0.341 \times 10^9/L$ [$554 \pm 341/\mu L$]; range, 0.118 - $1.363 \times 10^9/L$) than in those with AIDS with such results (mean $\pm$ SD CD4 cell count, $0.044 \pm 0.038 \times 10^9/L$; range, 0.013 - $0.104 \times 10^9/L$; $P < .001$).

Validation of the Sensitive/Less Sensitive Testing Strategy for Estimating HIV-1 Incidence

Comparisons of incidence estimates using the sensitive/less sensitive testing strategy with observed SFMHS cohort estimates are shown in Table 3. Despite the small number of persons at seroconversion risk in each follow-up period, point estimates for most 6-month follow-

up periods and for average incidence per follow-up period were similar.

In our validation in repeat blood donors, of 1 275 449 eligible donations, 16 donations tested HIV-1 positive. Of the 16, 10 were 3A11 reactive/3A11 nonreactive, giving an HIV-1 incidence estimate of 2.95 (95% CI, 1.14-6.53) per 100 000 per year, similar to incidence estimated from longitudinal observation in the same donors (2.60 [95% CI, 1.49-4.21] per 100 000 person-years) (G. Schreiber, REDS, written communication, September 5, 1997).

Applications of the Sensitive/Less Sensitive Testing Strategy

Of 2 717 910 first-time blood donations at 32 American Red Cross collection regions, 547 were positive and 69 were 3A11 reactive/3A11-LS nonreactive (Table 4). Incidence of HIV-1 in first-time blood donors was stable from mid-1993 through the end of 1996 (Table 4; $P = .17$).

From June 1996 through June 1997, in the Options Project, we enrolled 5 persons with reactive EIAs, negative or indeterminate Western blot results, and positive for HIV-1 RNA; all 5 were 3A11-

LS nonreactive. Also, we enrolled 17 persons who met a second study criterion of a documented negative HIV-1 antibody test result less than 6 months before a positive HIV-1 antibody test result at enrollment; all had 3A11-LS results consistent with early HIV-1 infection. Thus, the sensitive/less sensitive testing strategy alone would have identified all 17 persons seeking enrollment could document a negative result only in the previous 6 to 18 months but had evidence of early infection based on HIV-1-positive and 3A11-LS nonreactive results.

COMMENT

We developed a serologic testing algorithm that accurately diagnoses persons as having early HIV-1 infection many months after seroconversion is detectable by standard antibody assays. The ability to differentiate persons with early infection from those with later infection is a breakthrough for estimating HIV-1 incidence, for clinical care and research studies focused on early HIV-1 infection, and for guiding HIV prevention programs.

Based on our validation studies, the sensitive/less sensitive testing strategy can be expected to provide accurate national and local HIV-1 incidence estimates. Backcalculation has provided plausible descriptions of past HIV-1 incidence but estimates of recent HIV-1 incidence using it have great uncertainty.³⁶⁻³⁸ Also, change in the AIDS case definition in 1993³⁹ has further limited the use of backcalculation. The sensitive/less sensitive testing strategy, however, used in conjunction with HIV case surveillance, can provide minimum estimates of incidence in states with HIV surveillance,² and national estimates may be modeled from such data. At the local level, our sensitive/less sen-

sitive testing strategy can be used to provide timely estimates of incidence in cross-sectional studies in a variety of settings.⁸

To better understand HIV-1 transmission on a national level, we examined incidence trends in first-time blood donors at nearly three quarters of American Red Cross collection regions. Although AIDS cases in persons infected through heterosexual contact increased during the study period,² many were related to sexual transmission from injecting drug users. Incidence in blood donors is more likely to reflect persons at risk through heterosexual transmission from persons not at recognized risk. Our data suggest that there is no widespread increase in such heterosexual HIV-1 transmission in the United States.

Diagnosis of earliest stages of HIV-1 infection is important for clinical intervention and research.^{4,40-44} Patients with acute infection present with relatively nonspecific signs and symptoms that are often undiagnosed or misdiagnosed.^{6,7,45-46} An accurate diagnosis permits initiation of effective antiretroviral treatment to augment immunologic mechanisms that contain viral replication following primary viral dissemination.^{4,47-48} While plasma viral load decreases following seroconversion, in early infection, viral replication in lymphoid tissue remains as high as during acute infection, which provides a pathophysiologic basis for treating those with early infection as aggressively as those with acute infection.⁴⁹ The sensitive/less sensitive testing strategy would greatly assist in identifying persons for whom there is pathophysiologic and clinical^{50,51} evidence to support initiation of antiretroviral therapy. Also, in combination with viral load levels, knowledge of the timing of seroconversion can provide valuable information regarding a patient's prognosis.⁵²

Over a 1-year period in the Options Project, using the sensitive/less sensitive testing strategy, we could have increased enrollment by identifying seropositive persons who could not document an HIV-1-negative result in the preceding 6 months as having early infection. It is now included in criteria for study enrollment.

Those with early infection have recently engaged in high-risk behavior with a person with HIV-1 infection and may transmit the virus more efficiently than at other times during infection.^{53,54} Partner notification strategies using the sensitive/less sensitive testing strategy can be used to map the sexual or injecting drug use networks of a person with early HIV-1 infection⁵⁵ to identify groups in whom HIV-1 is being transmitted. Prevention activities can then be provided in the hope of interrupting ongoing viral transmission.

A limitation of the sensitive/less sensitive testing strategy is that a few persons with long-standing infection (0.4%) and late-stage AIDS (2%) could be misdiagnosed as having early infection. If one chose to use a higher OD cutoff than 0.75 for the 3A11-LS assay to identify additional seroconverting persons, the proportion misdiagnosed would be higher (Table 2, Appendix 3). However, CD4 cell counts in seroconverting persons in this study were similar to those in prior reports^{4,6} and higher than in AIDS patients with 3A11 reactive/3A11-LS nonreactive results. Thus, access to CD4 cell counts or clinical information on subjects will be needed when the sensitive/less sensitive testing strategy is used in care settings (Appendix 4). The strategy is most useful when first testing for HIV.

The sensitive/less sensitive testing strategy is inexpensive and reproducible and accurately identifies persons with early HIV-1 infection. It is useful at the population level for estimating HIV-1 incidence, at the clinical level for patient care and for identifying subjects with early infection for therapeutic trials and pathogenesis studies, and at the public health level for focusing and evaluating HIV prevention efforts.

APPENDIX 1

Using a Bonferroni procedure, CIs for incidence estimates using the sensitive/less sensitive testing strategy will reflect sampling variability of n_{dt} and uncertainty in mean time between seroconversions (T). To calculate 95% CIs (l_{95} , u_{95}) for incidence estimates, we obtained a 97.5% CI for n_{dt} using a Poisson assumption. The Bonferroni 95% CIs for incidence are $l_{95} = (n_{dt}/N)(365/T^+)(100)$, where n_{dt} is the lower 97.5% CI from the Poisson assumption, N is the number of HIV-1-negative persons plus the number with 3A11 reactive/3A11-LS nonreactive test results, and T^+ is the upper 97.5% CI for mean time between seroconversions; and $u_{95} = (n_{dt}^+/N)(365/T^-)(100)$, where n_{dt}^+ is the upper 97.5% CI from the Poisson assumption, and T^- is the lower 97.5% CI for mean time between seroconversions. For a 3A11-LS cutoff of 0.75, we estimate $T^- = 108$ days and $T^+ = 154$ days; for cutoff of 1.0, we estimate $T^- = 130$ days and $T^+ = 205$ days.

APPENDIX 2

The formula for calculating HIV-1 incidence using the sensitive/less sensitive testing strategy must be modified to account for multiple donations from the same subject. Incidence can be calculated using $(n_{dt}/N_d)(365/\gamma)(100)$, where n_{dt} is number of 3A11 reactive/3A11-LS nonreactive specimens detected in eligible donations, N_d is number of eligible donations

given (excluding the initial, seronegative donation establishing eligibility for the sample), and $\gamma = E[\int_0^T S(x)dx]$, where E denotes expected value with respect to interdonation intervals ΔT and $S(x)$ is proportion of 3A11 reactive/3A11-LS nonreactive results x days after 3A11 seroconversion as shown in Figure 3, left. This equation has the same form as that in the text, but with T replaced by γ . To derive this result, observed incidence is obtained by dividing number of seroconverting persons donating repeatedly by person-time of observation. To use the sensitive/less sensitive testing strategy, we replaced number of seroconverting persons by number of persons who were 3A11 reactive/3A11-LS nonreactive, divided by probability that seroconverting persons would be observed as being 3A11-LS nonreactive at time of first 3A11 reactive donation. Following Satten,⁵⁶ this probability is given by $E[\int_0^T S(x)dx]/\Delta T$ where ΔT denotes average interdonation interval. Because person-time of observation can be written as $N_d\Delta T$, we obtain the desired equation. To estimate γ , we used the empirical distribution of interdonation intervals from REDS to compute the required expected value. Due to the large number of interdonation intervals contributing to this empirical distribution, bootstrap CIs for γ assumed that the only source of variability was $S(x)$.

APPENDIX 3

Selection of a cutoff for the 3A11-LS assay for studies of incidence represents a balance of sample size with uncertainty of incidence estimate and proportion of persons with AIDS possibly misclassified as having early infection. Smaller sample sizes can be achieved by raising the 3A11-LS assay cutoff, but at the cost of increasing incidence estimate uncertainty and increasing proportion of persons with possible AIDS misclassification (Table 2). When using the sensitive/less sensitive testing strategy for clinical purposes and prevention activities, an OD cutoff as high as 2.00 for the 3A11-LS assay may be acceptable since follow-up samples can be obtained to confirm early infection using CD4 cell count or by showing a progressive increase in reactivity on the 3A11-LS assay.

APPENDIX 4

To determine upper limit of acceptable prevalence of persons with AIDS in a sample, one can choose arbitrarily an acceptable percentage of the 3A11 reactive/3A11-LS nonreactive specimens coming from persons with AIDS. When using a cutoff of 0.75 for the 3A11-LS assay, to ensure that fewer than k percentage of specimens testing 3A11 reactive/3A11-LS nonreactive are due to late-stage AIDS patients, $p_{AIDS} < k/(100 - k)(T/365)$

($i/0.024$), where p_{AIDS} is the prevalence of AIDS cases in the sampled population (as a percentage); k is the acceptable percentage of 3A11 reactive/3A11-LS nonreactive samples attributable to AIDS patients; T is mean time between seroconversion on the 3A11 and 3A11-LS assays; and i is HIV-1 incidence in percentage per year. For example, if $k = 10$, $T = 129$ days, and $i = 1\%$, then for fewer than 10% of 3A11 reactive/3A11-LS nonreactive samples to come from AIDS patients, p_{AIDS} must be less than 1.6%.

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and Critical Control Point principles to the processing of these foods to control potential contamination.³ Until the final rule is implemented, the FDA requires packaged juice that has not been processed to prevent, reduce, or eliminate pathogens that may be present to bear a warning statement informing consumers of the potential risk of these products.³

We hope that the final implementation of the FDA's new rules will result in the production of safe product. Until that time, consumers, in particular those at greatest risk of developing serious illness from enteric pathogens (eg, children, the elderly, and persons with weakened immune systems), should heed warning labels now being placed on unpasteurized juice products.³ Those wanting to reduce their risk for illness should drink only pasteurized juices.

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Is Osteopathic Medicine "Alternative"?

To the Editor: Dr McPartland¹ raises an interesting issue in criticizing my book on alternative medicine for excluding osteopathy. Is osteopathic medicine "alternative"?

Contrary to McPartland's belief, but according to the American Osteopathic Association, mainstream training and licensure requirements, and the American Medical Association, which includes doctors of osteopathy among its members, osteopathic medicine is decidedly not alternative. Students of osteopathic medicine train for the same length of time and according to the same standards applied in allopathic medical schools, often must pass the same tests and licensing examinations, and, like doctors of medicine, may be licensed for the full practice of medicine in all 50 states. No other group is so trained or permitted.^{2,3}

Further, osteopathic medical school accreditation is recognized by the US Department of Education. Applicants to schools of both allopathic and osteopathic medicine are required to take the medical college admission test.

Alternative medicine by definition is unproved, and it exists primarily outside mainstream medicine. If it were proved and fully accepted, it would not be alternative. The thorough integration of osteopathic medicine into the mainstream suggests that the views of McPartland, who is the director of an alternative medicine clinic, are not shared by most of his fellow practitioners.

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2. American Osteopathic Association Web site. Available at: <http://www.aoa-net.org>. Accessed April 15, 1999.

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In Reply: Dr Cassileth's question "Is osteopathic medicine alternative?" currently cleaves the osteopathic community. This issue has been addressed in a recent survey¹ and numerous editorials.^{2,3} Cassileth has her finger on the pulse of the "allopathic wing" of the American Osteopathic Association. Verily, American Osteopathic Association Executive Director R. Draba summarized his opinion in a letter to me: "Osteopathic medicine is as American as apple pie and Chevy trucks. It's mainstream medicine . . . there's nothing alternative about osteopathic medicine" (written communication, 1994).

But if we define alternative medicine as "any medical intervention not taught widely at allopathic medical schools, nor generally available at hospitals,"^{4,5} then osteopathic manipulative treatment qualifies as alternative. Inferentially, a physician who uses osteopathic manipulative treatment can be defined as an alternative practitioner. This label should not disturb physicians familiar with the writings of A. T. Still,⁶ the founder of osteopathy, who offered his reform movement as a rational alternative to the practice of 19th-century medicine.

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CORRECTIONS

Incorrect Number: In the Original Contribution entitled "Early Health Effects of the Emerging Tobacco Epidemic in China: A 16-Year Prospective Study" published in the November 12, 1997, issue of THE JOURNAL (1997;278:1500-1504), an incorrect number appeared in the abstract. In the fifth line under "Results," the number 22 in the confidence interval should be 2.2.

Incorrect Wording: In the Original Contribution entitled "New Testing Strategy to Detect Early HIV-1 Infection for Use in Incidence Estimates and for Clinical and Prevention Purposes" published in the July 1, 1998, issue of THE JOURNAL (1998;280:42-48), there was incorrect wording in the abstract. On page 42, under "Results," the first sentence should read as follows: "Estimated mean time from being 3A11 reactive/3A11-LS nonreactive to being 3A11 reactive/3A11-LS reactive was 129 days (95% confidence interval [CI], 109-149 days)."