Detection of Early Sero-Conversion HIV Infection Using the INSTI™ HIV-1 Antibody Point-of-Care Test

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Abstract: We compared the INSTI™ HIV-1 Antibody Point-of-Care (POC) Test to laboratory-based tests for detection of early sero-conversion (i.e. acute) HIV infections. Fifty-three (53) individuals with early HIV infection, (i.e. 3rd generation anti-HIV EIA non-reactive or reactive, HIV-1 Western Blot non-reactive or indeterminate and HIV-1 p24 antigen reactive) were tested by INSTI™. The INSTI™ test was reactive for 34/49 (sensitivity 69.4%; 95% confidence interval 54.6-81.8%) early-infected individuals whose laboratory-based 3rd generation HIV EIA test was reactive. Four (4) were non-reactive by both the laboratory-based EIA and INSTI™ tests, but were p24 antigen reactive. The INSTI™ POC test performs well compared with other POC tests for the detection of early sero-conversion HIV infection, but it may miss 20% to 30% of those detected by laboratory-based 3rd generation anti-HIV tests. Both POC and laboratory-based anti-HIV tests will fail to detect a proportion of infected individuals in the first weeks after infection.

Keywords: HIV rapid tests, point of care, early sero-conversion, primary HIV infection.

FINDINGS

Acute HIV infections contribute significantly to the risk of secondary infections since acutely-infected individuals (i.e. during the early sero-conversion period) have very high viral loads and many are unaware of their infection [1, 2]. Current standard-of-care 3rd generation anti-HIV tests are inadequate to identify a proportion of HIV-infected individuals during the early sero-conversion phase and additional tests to identify HIV p24 antigen or HIV RNA are required [3, 4]. Widespread application of HIV testing and early diagnosis are important to identify HIV-infected individuals to support effective prevention and care. In order to provide counseling regarding the accuracy of the various tests and for planning HIV testing programs, it is important to know the relative effectiveness of HIV point-of-care (POC) *vs* laboratory-based tests for detecting early sero-conversion HIV infections. In Canada, the INSTI™ HIV-1 Antibody Test (bioLytical Laboratories, Richmond BC) is the only licensed POC HIV test and its overall sensitivity and specificity are similar to laboratory-based 3rd generation enzyme immunoassay (EIA) tests [5]. The manufacturer makes no specific sensitivity claims regarding the early sero-conversion phase of HIV infection, but data from testing of 25 sero-conversion panels are available [5]. For 15 panels, the INSTI™ test became reactive on the same bleed, for seven panels one bleed later and for one panel two bleeds later than the referent 3rd generation laboratory EIA. For two panels, the INSTI™ test was non-reactive on the final bleed in the panel. The sensitivities of other POC tests for detection of early sero-conversion HIV infection have been reported to be lower than for laboratory-based tests [3, 6-9].

The objective of this study was to assess the sensitivity of the INSTI™ test compared to laboratory-based HIV tests, using residual sera collected from individuals with early sero-conversion HIV infection. The study period was Feb 2006 to Oct 2008. Presumptive early sero-conversion HIV infection was based on laboratory criteria, i.e. 3rd generation anti-HIV EIA (Siemens ADVIA™ Centaur HIV-1/O/2) non-reactive or reactive, HIV-1 Western Blot (WB) (BioRad Genetic Systems HIV-1 Western Blot) non-reactive or indeterminate and HIV-1 p24 antigen (Biomérieux Vironostika HIV-1 Antigen) reactive with confirmation by neutralization or by HIV nucleic acid testing (NAT) (Roche AMPLICOR™ HIV-1 DNA Test v. 1.5). WB interpretation criteria were: non-reactive (no bands are present); indeterminate (one or more bands are present but the blot does not meet reactive test criteria); reactive [at least two major bands (gp160 and/or gp120; gp41 or p24) must be present]. Cases were excluded if: there was insufficient residual serum for testing; the initial presumptive early sero-conversion HIV result was not confirmed by follow-up WB, NAT or physician-reported viral load result; or individuals were known to have advanced HIV disease at diagnosis based on receipt of an AIDS case report within 12 months of a presumptive early sero-conversion HIV result. All subjects gave informed consent for HIV testing. The study was approved by the University of British Columbia Clinical Ethics Review Board.

Sixty-one (61) presumptive early sero-conversion HIV infections were identified, of which eight were excluded (four had insufficient residual serum for testing, two were cases which had no follow-up confirmatory WB testing, and two had an AIDS case report received within 12 months of

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the presumptive early sero-conversion HIV result). Thus, specimens from 53 individuals were available for analysis. In addition, 10 serum samples from HIV-uninfected individuals (laboratory 3rd generation EIA non-reactive) were tested, but there was no intent to evaluate the specificity of the INSTI™ assay, which has already been established [5].

Demographic characteristics of the early sero-conversion HIV cases were: 85% male; mean age 39 years; 95% HIV-1 sub-type B; 71% Caucasian; 59% men who have sex with men (MSM), 25% injection drug user (IDU), and 20% heterosexual, non-IDU (individuals may report more than one exposure category). The demographics of the early sero-conversion HIV cases were not significantly different from those of 926 other newly-identified HIV infections diagnosed during the study period, except that the early sero-conversion cases were more likely to be MSM [unadjusted odds ratio 1.71; 95% confidence interval (CI) 1.01-2.89]. Of the 53 early sero-conversion HIV specimens, four were laboratory EIA non-reactive but p24 antigen reactive and 49 were laboratory EIA reactive. The laboratory EIA protocol incorporates a 10% grey zone; specimens falling within the grey zone on initial testing are re-tested, and, if still in the grey zone are subjected to confirmatory testing. All residual initial specimens and any available follow-up specimens were tested by INSTI™. Each INSTI™ test was interpreted by three technologists; in case of disagreement, the consensus interpretation was used. Interpretation criteria for the INSTI™ test are: invalid (the control spot is not visible); non-reactive (the control spot is visible but there is no visible reaction on the test spot); indeterminate (the control spot is visible and a faint background ring appears on the test spot); reactive (the test spot and control spot are both visible).

The 10 laboratory EIA non-reactive specimens from uninfected individuals were non-reactive by INSTI™. As would be expected, the four laboratory EIA non-reactive, p24 antigen reactive early sero-conversion specimens were also non-reactive by INSTI™. Even when incorporating the 10% grey zone for the laboratory EIA, all four were non-reactive. Testing for early sero-conversion infection would have been specifically requested for these individuals (e.g. following a risk event or because of symptoms compatible with acute HIV infection); all four individuals became reactive by INSTI™ and the laboratory EIA on follow-up testing (Table **1**). Of the 49 laboratory EIA reactive early sero-conversion specimens, the INSTI™ test was reactive for 34/49 (69.4%), indeterminate for 5/49 (10.2%) and non-reactive for 10/49 (20.4%). Thus, the sensitivity of INSTI™ compared to the laboratory EIA for detection of early sero-conversion HIV infection in this population was 69.4% (95% CI 54.6-81.8%). There was no statistically significant association between an individual's exposure category and a reactive INSTI™ result.

The five INSTI™ indeterminate initial specimens would normally be submitted for confirmatory laboratory testing, as recommended by the manufacturer, and this would have been expected to confirm early sero-conversion HIV infection. Thus, in practice, the initial INSTI™ test results would likely have led to the correct screening diagnosis for 39/49 (79.6%) of these early infections. Of the 15 INSTI™ indeterminate and non-reactive initial specimens, the INSTI™ test became reactive on 13/13 (100%) individuals who had follow-up specimens at a later date (Table **1**). The other two individuals had follow-up serology which confirmed HIV infection, but there was insufficient residual serum available for INSTI™ testing.

Louie*, et al.* [6] reported that of US-licensed rapid and POC HIV tests, the most sensitive assay detected 11/42 (26.2%) early sero-conversion infections. These results may not be directly comparable to those in our study, as the specimens examined by Louie*, et al.* were selected from acute infections diagnosed by pooled NAT testing of pre-screened 1st generation EIA non-reactive specimens, and might reflect sampling earlier during infection. Our results are consistent with the findings of Giles*, et al.* [7], who reported that of 13 simple/rapid HIV test devices, none detected sero-conversion earlier than the most sensitive laboratory EIAs and four performed similarly. Laforgerie, *et al.* [8] recently reported that for eight CE (European Community)-approved rapid disposable tests, including the INSTI™ test, earliest detection among three sero-conversion panels was variable. The INSTI™ test detected two panels at the same time and for one panel, one bleed later than the earliest detection by any rapid test. It is possible that POC test sensitivity is related to the input sample volume used for testing. The INSTI™ test utilizes 50µL of sample, a larger volume than many other rapid/POC tests.

This study has several limitations. Interpretation of results of POC tests performed by trained technologists in a laboratory setting may not be directly generalizable to a clinical setting. It is recognized that necessary quality assurance practices must be in place for accurate POC testing and that non-laboratory or inexperienced health care providers may have difficulties in test result interpretation [10]. A potential source of study bias is that the technologists reading the tests were not blinded to the HIV sero-status of the samples; however, three technologists interpreted each result identically, except for one sample which was read as indeterminate by one technologist and non-reactive by the other two. It is also recognized that individuals with advanced HIV disease may have HIV serological result patterns similar to acute cases, i.e. 3rd generation EIA reactive, p24 antigen reactive, WB indeterminate. None of the 53 early sero-conversion cases had an associated AIDS report within 12 months of diagnosis; however, due to incomplete and delayed AIDS reporting, it might be feasible that some individuals with advanced HIV infection were mis-classified as early sero-conversion. Given that only residual specimens of known sero-status were assessed, we could not identify individuals with early sero-conversion HIV infection who might have had a reactive INSTI™ test at the same time as, or prior to, a non-reactive laboratory EIA, as has been reported for other POC tests [6]. This analysis involved residual serum, whereas in clinical practice, the INSTI™ POC test uses whole blood which has been shown to provide equivalent results [5]. The study was not designed to assess the overall specificity of the INSTI™ test nor the

Table 1. Results of Follow-Up Testing of INSTI™ Non-Reactive or Indeterminate Early HIV Sero-Conversion Specimens

Case #	Initial Specimens[a]			Follow-Up Specimens[b]		
	Siemens ADVIA Centaur EIA (Signal/Cutoff)	Siemens ADVIA Centaur EIA Result	INSTI™ Result	Siemens ADVIA Centaur EIA Result	INSTI™ Result	Days From Initial to INSTI™ Reactive Followup Specimen[d]
3	0.8	NR	NR	R	R	49
6	0.8	NR	NR	R	R	251
18	<0.05	NR	NR	R	R	16
33	<0.05	NR	NR	R	R	15
2	1.9	R	Indet	NT	R	29
4	23.2	R	Indet	R	R	88
29	18.5	R	Indet	R	R	16
30	17.3	R	Indet	R	R	13
51	1.0	R	Indet	NT	R	20
7	4.0	R	NR	R	NSQ	
9	10.4	R	NR	R	R	42
11	8.1	R	NR	R	R	73
21	>50	R	NR	R	R	26
25	1.3	R	NR	R	NSQ	
31	41.0	R	NR	R	R	8
37	13.8	R	NR	R	R	8
49	2.5	R	NR	R	R	22
57	1.2	R	NR	R	R[c]	12
60	3.3	R	NR	R	R	7

NR=non-reactive; R=reactive; Indet=indeterminate; NSQ=insufficient quantity; NT=not tested.
[a]All specimens were HIV-1 p24 antigen confirmed reactive.
[b]All specimens were HIV-1 Western Blot positive.
[c]A follow-up specimen collected 7 days after the initial specimen was INSTI™ indeterminate.
[d]Mean and median times from initial to INSTI™ reactive follow-up specimen are 41 and 20 days, respectively.

specificity amongst individuals who present with possible HIV sero-conversion symptoms. The study was carried out using a single batch of INSTI™ tests and the possibility of lot-to-lot variability for the detection of early sero-conversion infections cannot be excluded.

In summary, although most early sero-conversion HIV infections in our study were detected by INSTI™ at the time of the initial specimen draw, 20-30% of early-infected individuals would have been missed. At least one 4th generation POC test, which is able to detect both anti-HIV and p24 antigen, is available and others are in development. Until 4th generation POC tests are widely adopted and are shown to reliably detect early sero-conversion HIV infection, combining POC testing with laboratory-based NAT or 4th generation EIA testing could improve detection of early sero-conversion HIV infections in high incidence settings, and this has been recommended by others [3, 4].

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CONFLICT OF INTEREST

The authors declare that they have no conflicts.

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