Results from a Multi-Centre Canadian Clinical Trial of a Rapid HIV Antibody Test for Use in Point-of-Care, Clinical and Laboratory Settings

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Background

All diagnostic and blood screening HIV testing devices in Canada are regulated through the Bureau of Medical Devices, Health Canada. Currently there are no rapid HIV testing devices approved for use in point-of-care (POC) settings in Canada.

The INSTI HIV-1/HIV-2 Antibody test (Biolytical Laboratories Inc., Richmond, BC.) is a rapid (60 second) *in vitro* membrane-based qualitative test for the detection of antibodies to Human Immunodeficiency Viruses Types 1 and 2 (collectively referred to as HIV-1/2) in human whole blood, serum or plasma.

The purpose of this prospective Clinical Study is to evaluate the performance characteristics of the INSTI HIV1/2 Rapid Antibody Test Kit in comparison to "gold standard" licensed laboratory-based tests.

Objective

We wished to assess the performance of the INSTI Rapid HIV-1/HIV-2 Antibody Test Kit through a large scale prospective clinical trial, conducted in accordance with the Medical Devices Regulations, Health Canada, for approval for use in POC, clinical and laboratory settings.

Study Design

Intended Patient/Sample Population:

• A prospective, cross-sectional, voluntary-testing population of **2,500** consenting patients of unknown HIV status, from 3 provinces (BC, Alberta, Ontario), representing all risk levels, plus **1,000** combined archived and prospective samples from patients with confirmed HIV-1 infection (commencing Sept, 2003).
•Up to 300 world-wide confirmed HIV-2 frozen, archived plasma/serum samples.
•25 commercial seroconversion panels, to measure early antibody detection.

Study Protocol:

•Patients were enrolled in the study through participating POC sites including Medical, STD, Family Planning, Anonymous Testing and hospital-based Immunodeficiency Clinics in Vancouver, Calgary and Toronto. Patients from the Vidus (Vancouver injection drug user study) and Vanguard (Vancouver gay men's study) cohorts were also included.
•Finger-stick whole blood was collected and tested with the INSTI kit at the POC sites. Matching venous blood was also collected in EDTA tubes from all patients and forwarded to the Provincial Public Health Laboratories (PHL). A subset of matching red-top blood samples (for serum) was collected in selected clinics.
• At the PHL, INSTI testing was carried out on matching EDTA blood as well as plasma and serum, within 48 hours of collection. Gold standard serology testing by the PHL test of record (Abbott AxSym MEIA HIV 1/2 GO in all PHL sites) was conducted on serum and/or plasma, with discordant and/or indeterminate results resolved via supplemental EIA, Western Blot, and p24Ag as necessary.
•Three production lots of INSTI were tested.
•All staff performing INSTI were trained and validated by Biolytical prior to testing patient samples.
•Patients were not provided with results of the INSTI rapid test.

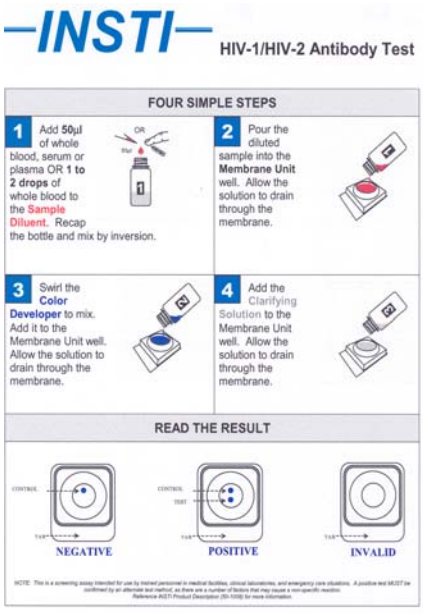
Methods

•The **INSTI HIV-1/HIV-2** test cassette consists of a synthetic filtration membrane positioned atop an absorbent material within a plastic cartridge. The membrane has been specifically treated with HIV-1 and HIV-2 recombinant proteins, which react with HIV-1 and/or HIV-2 antibodies in the specimen to produce a distinct visual signal on the membrane.

•The membrane also includes a human IgG-capture control which consists of a protein-A treated spot capable of binding IgG antibodies normally present in blood and blood components. If the control spot does not appear, the test is considered invalid.

•All INSTI results were compared to licensed gold standard test kit results. The INSTI procedure is illustrated in Figure 1:

Figure 1:



Results

The following tables summarize the aggregate results from the prospective, cross-sectional, voluntary testing population of unknown HIV status as well as those with known HIV-1 infection tested in POC and laboratory settings through June 30, 2004:

Table 1: POC INSTI results compared to PHL-based Abbott AxSym, n=3467

	Abbott Axsym	
	Positive	Negative
POC INSTI Positive	816	18[1]
POC INSTI Negative	15[2]	2448
POC INSTI Invalid	27[3]	143

1 All were HIV antibody negative by confirmatory testing (combination of Western Blot, supplemental EIA, or p24Ag), ie INSTI false positive.
2 11/15 were negative by confirmatory testing, ie AxSym false positive. The remaining 4 were confirmed as HIV antibody positive, ie INSTI false negative.
3 All were Western Blot positive. Invalid INSTI results were not used for calculations of sensitivity and specificity.

Sensitivity of POC INSTI: 99.5% (816/820, 95% CI: 98.8 - 99.8%); **PPV: 97.8%**

Specificity of POC INSTI: 99.3% (2459/2477, 95% CI: 98.9 - 99.5%); **NPV: 99.8%**

Table 2: PHL INSTI on whole blood (EDTA) compared to PHL-based Abbott AxSym, n=3462

	Abbott Axsym	
	Positive	Negative
Blood INSTI Positive	797	16[1]
Blood INSTI Negative	14[2]	2620
Blood INSTI Invalid	2	13

1 All were HIV antibody negative by confirmatory testing (combination of Western Blot, supplemental EIA, or p24Ag), ie INSTI false positive.
2 11/14 were negative by Western Blot ie. AxSym false positive. The remaining 3 were confirmed as HIV antibody positive, ie INSTI false negative.

Sensitivity of Blood INSTI: 99.6% (797/800, 95% CI: 98.9 - 99.9%); **PPV: 98.0%**

Specificity of Blood INSTI: 99.4% (2631/2647, 95% CI: 99.0 - 99.6%); **NPV: 99.9%**

Table 3: PHL INSTI on plasma compared to PHL-based Abbott AxSym, n=3462

	Abbott Axsym	
	Positive	Negative
Plasma INSTI Positive	798	2[1]
Plasma INSTI Negative	14[2]	2640
Plasma INSTI Invalid	1	7

1 All negative by Western Blot, ie. INSTI false positive.
2 11/14 Western Blot negative (ie AxSym false positive), 3/14 Western Blot positive, ie INSTI false negative.

Sensitivity of Plasma INSTI: 99.6% (798/801, 95% CI: 98.9 - 99.9%); **PPV: 99.7%**

Specificity of Plasma INSTI: 99.9% (2651/2653, 95% CI: 99.7 - 100%); **NPV: 99.9%**

Table 4: PHL INSTI on serum compared to PHL-based Abbott AxSym, n=1384

	Abbott Axsym	
	Positive	Negative
Serum INSTI Positive	361	1[1]
Serum INSTI Negative	3[2]	1016
Serum INSTI Invalid	0	3

1 negative by Western Blot, ie. INSTI false positive.
2 All positive by Western Blot, ie INSTI false negative

Sensitivity of Serum INSTI: 99.2% (361/364, 95% CI: 97.6 - 99.7%); **PPV: 100%**

Specificity of Serum INSTI: 99.9% (1016/1017, 95% CI: 99.4 - 100%); **NPV: 99.7%**

Seroconversion Panels

Relative sensitivity of INSTI compared to approved EIA methods for detection of antibodies in Commercial (BBI) Seroconversion Panels (n=25).

INSTI Performance	Number of Panels
Detected earliest bleed of panel	15
Within 1 bleed of earliest EIA positive	7[1]
Within 2 bleeds of earliest EIA positive	1[2]
Unknown	2[3]

1 INSTI positive on panel samples collected 3 - 8 days following earliest EIA positive.
2 INSTI positive on panel sample collected 7 days after earliest EIA positive.
3 the last bleed in the panel was positive by at least 1 EIA, but negative by INSTI.

HIV-2 Detection, n=49

•All 49 frozen (-20C) serum samples were confirmed positive for HIV-2 antibodies by Western Blot (New Lav Blot HIV-2 (Biorad).

•49/49 were positive with the INSTI HIV-1/HIV-2 rapid antibody test (100% sensitivity).

•All INSTI and HIV-2 testing was conducted at the Laboratoire de Virologie, C.E.R.V.I., G.H. Pitie-Salpetriere, Paris, France.

Discussion and Conclusions

•The INSTI HIV-1/HIV-2 rapid antibody test in POC settings as well as laboratory testing sites was equivalent to the Health Canada approved laboratory test of record (Abbott AxSym GO) in overall sensitivity, specificity, and early antibody detection. Performance characteristics of INSTI were highly concordant across matching finger-stick blood, venous whole blood (EDTA), plasma and serum. There was no INSTI lot-to-lot variation in performance observed. Work is continuing for HIV-2 performance characteristics.

•Invalid results were more frequent at POC sites, and tended to be the result of sub-optimal amounts of blood collected with the disposable transfer pipette included in the kit. This is evidence that the INSTI test device will only work with the addition of adequate amounts of human IgG, providing a high degree of patients' safety in validity of results.

British Columbia Centre for Excellence in HIV/AIDS