CANADIAN CLINICAL TRIAL RESULTS

Background Results

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 Virus Type 1 in human whole blood, serum or plasma.

The purpose of this prospective Clinical Study is to evaluate the performance characteristics of the INSTI™ HIV-1/HIV-2* Rapid Antibody Test Kit in comparison to "gold standard" licensed laboratory-based tests.

Objective

We wished to assess the performance of the INSTI™ 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/HIV-2* infection (commencing Sept, 2003).
- 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™ kits 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™ HIV-1/HIV-2* Antibody Test.**

Methods

- INSTI™ HIV-1/HIV-2* Antibody 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/HIV-2* recombinant proteins, which react with HIV-1/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.

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

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=3507

Abbott Axsym

	Positive	Negative	Indeterminate
POC INSTI™ Positive	**817**	**18**[1]	**1**[2]
POC INSTI™ Negative	**13**[3]	**2478**	**1**[4]
POC INSTI™ Invalid	**38**	**141**	**0**

1. All 18 are considered INSTI POC false positive.
2. Sample B1B0031. Possible early seroconversion.
3. 10/13 considered Axsym false positive.
4. Sample initially positive on Axsym, but negative 2X on repeat. WB shows weak bands at p24 and p65 only.

Sensitivity of POC INSTI™: 99.6% (817/820, 95% CI: 98.9 - 99.9%); **PPV**: 97.8%
Specificity of POC INSTI™: 99.3% (2488/2506, 95% CI: 98.9 - 99.5%); **NPV**: 99.5%

Table 2: PHL INSTI™ on whole blood (EDTA) compared to PHL-based Abbott AxSym, n=3481

Abbott Axsym

	Positive	Negative	Indeterminate
POC INSTI™ Positive	**831**	**9**[1]	**1**[2]
POC INSTI™ Negative	**15**[3]	**2611**	**1**[4]
POC INSTI™ Invalid	**10**	**3**	**0**

1. All 9 are considered INSTI false positive
2. Sample B1B0031, possible early seroconversion.
3. 10/15 considered Axsym false positive
4. Sample initially positive on Axsym, but negative 2X on repeat. WB shows weak bands at p24 and p65 only.

Sensitivity of POC INSTI™: 99.4% (831/836, 95% CI: 98.6 – 99.7%); **PPV:** 98.9%
Specificity of POC INSTI™: 99.7% (2621/2630, 95% CI: 99.4 – 99.8%); **NPV:** 99.9%

Table 3: PHL INSTI™ on plasma compared to PHL-based Abbott Axsym, n=3479

Abbott Axsym

	Positive	Negative	Indeterminate
POC INSTI™ Positive	**834**	**1**[1]	**0**
POC INSTI™ Negative	**14**[3]	**2627**	**2**[3]
POC INSTI™ Invalid	**1**	**0**	**0**

1. Sample B3B 0240, considered INSTI blood and plasma false positive. See Table of Discordant Results.
2. 10/14 considered Axsym false positive
3. Sample B1B0031, possible early seroconversion. Sample B1B0568: Axsym initially positive, but neg 2X on repeat. WB shows weak bands at p24 and p65 only.

Sensitivity of POC INSTI™: 99.5% (834/836, 95% CI: 98.8 – 99.8%); **PPV:** 99.9%
Specificity of POC INSTI™: 100% (2627/2628, 95% CI: 99.8 – 100%); **NPV:** 99.9%

Table 4: PHL INSTI™ on serum compared to PHL-based Abbott Axsym, n=1346

Abbott Axsym

	Positive	Negative	Indeterminate
POC INSTI™ Positive	**392**	**0**	**0**
POC INSTI™ Negative	**4**[1]	**949**	**0**
POC INSTI™ Invalid	**1**	**0**	**0**

1. Samples considered early seroconversion; INSTI positive on whole blood

Sensitivity of POC INSTI™: 99.5% (392/396, 95% CI: 97.4 – 99.6%); **PPV:** 100%
Specificity of POC INSTI™: 100% (949/949, 95% CI: 99.6 – 100%); **NPV:** 99.6%

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

INSTI™	Number of Panels
Detected earliest bleed of panel	**14**
Within 1 bleed of earliest EIA positive	**8**[1]
Within 2 bleed of earliest EIA positive	**1**[2]
Unknown	**2**[3]

1. INSTI positive on panel samples collected 3-8 days following the earliest positive EIA
2. INSTI positive on panel sample collected 7 days after the earliest positive EIA
3. The last bleed in the panel was positive by at least 1 EIA, negative by INSTI. Anti-HIV-1 Seroconversion Panels PrB937 and PRB938

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.