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Evaluation of the accuracy and ease of use of a rapid HIV-1 Antibody Test performed by untrained operators at the point of care☆

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ABSTRACT

Background: For broader utilization of rapid HIV antibody assays in point-of-care (POC) settings, methods should be simple enough to be performed with accuracy by untrained test providers, using only the test manufacturer's written instructions.
Objective: To demonstrate that the INSTI HIV-1 Antibody Test is simple and accurate enough to be successfully run by untrained operators in a POC setting.
Study design: A prospective study was conducted to compare the results of the FDA-cleared, INSTI HIV-1 Antibody Test (INSTI, bioLytical Laboratories Inc., Richmond, BC, Canada) used by untrained operators on finger-stick whole blood to results obtained by trained laboratory professionals using FDA-cleared comparator methods (CM) on matching venous blood. A total of 1388 subjects were recruited into the study in three diverse US POC sites. One central laboratory was used for CM testing. Untrained operators and experienced laboratory professionals also conducted a study on prepared plasma specimens to compare limit of detection (LoD) abilities.
Results: Of the 517 HIV positive subjects (34 new positives and 483 known positives) the concordance between INSTI performed by untrained operators and CM performed by trained laboratory professionals was 100% (95% CI = 99.3–100%). Concordance for HIV negative results (n = 871) was 99.8% (95% CI = 99.2–99.9%). There were no significant differences in INSTI limit of detection between untrained operators and laboratory professionals.
Conclusions: Untrained operators with no laboratory background were able to perform and interpret the results of INSTI on finger-stick blood and LoD specimens with a high degree of accuracy by following only the manufacturer's written instructions.

1. Background

The US Centers for Disease Control and Prevention estimates that up to 25% of persons living with human immunodeficiency virus (HIV) in the US are unaware of their infection and therefore are not linked to treatment and care [1]. Moreover, the World Health Organization estimates that as many as 80% of individuals living with HIV in the developing world have not been diagnosed [2], ultimately perpetuating the epidemic. As a result, new global initiatives designed to seek, test, treat and retain individuals infected with HIV have emerged. Treatment as prevention strategies, developed following the HPTN 052 study on discordant couples [3] have proven that effective treatment can dramatically reduce transmission of HIV [4]. Widespread HIV testing scale-up is at the core of these global initiatives to halt the spread of HIV infection, and as a result, The U.S. Preventive Services Task Force (USPSTF) is poised to release recommendations on screening for human HIV infection that will endorse the routine testing of adults and adolescents [5] a position first adopted by the Centers for Disease Control and Prevention (CDC) in 2006 [6].

Rapid HIV test methods are widely utilized throughout the world, and multi-test algorithms have been developed to optimize their use [7,8]. The accuracy of many of these rapid HIV antibody detection tests is comparable to laboratory-based antibody

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detection assays [9,10] These rapid test methods provide affordable, accurate and accessible aids to the diagnosis of HIV infection in low resource settings as well as in developed country programs [11,12]. Diagnostic HIV testing in the US, Canada, and other global regions is often conducted in clinical and non-clinical point-of-care (POC) settings such as outpatient clinics, medical offices, Voluntary Counseling and Testing (VCT) Centers, testing outreach/community services, and sexual health programs [13] These POC testing programs tend to rely upon the use of samples of convenience, i.e. oral fluid and fingerstick blood, and rapid test technologies that are frequently performed by non-professional, untrained operators. In addition, rapid testing methods are being increasingly utilized in supervised and unsupervised self-testing and over-the counter (OTC) applications [14]. It is therefore important to demonstrate that rapid test procedures and interpretation of results in these settings are simple enough to be used by trained and untrained intended use operators without increased errors in test performance and/or interpretation of results.

Due to the emergence of POC testing as an accepted standard of practice for HIV test delivery in many global settings, regulatory bodies such as the US Food and Drug Administration Center for Devices and Radiological Health (CDRH) are placing greater emphasis on intended users and use of prospective patient specimens in studies testing a device for overall safety and ease of use in the hands of untrained operators [15,16].

2. Objective

The objective of this study was to determine that the INSTI test procedure and interpretation of results in POC settings are simple enough to be performed by untrained intended use operators without increased risk for error. To demonstrate this, the results of the INSTI HIV-1 Antibody Test conducted by untrained operators on finger-stick blood from 1388 subjects with unknown and known-HIV positive status were compared to the results of FDA-approved Comparator Methods (CM) performed by laboratory professionals on matching venous blood.

3. Study design

INSTI was to be performed by untrained operators on fingerstick blood for all study subjects at the time of enrollment by following the package insert provided with the test kits and using a quick reference guide provided by the manufacturer. The INSTI™ HIV-1 Antibody Test is a manual, visually read, flow-through immunoassay for the qualitative detection of HIV-1 antibodies in human blood obtained from fingerstick or venipuncture, and plasma. The test consists of a nitrocellulose filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI™ Membrane Unit. The membrane has been spotted with HIV-1 and HIV-2 recombinant proteins, which react with HIV antibodies in the specimen. The test is intended for use by personnel in point of care and laboratory situations to aid in the diagnosis of HIV-1 infections.

Table 1
Baseline subject demographics for the 1418 eligible subjects:.

Gender	*N* (%)
Female	409 (28.8%)
Male	1009 (71.2%)
Race	
American Indian/Alaskan Native	33 (2.3%)
Asian	6 (0.4%)
Black/African American	594 (41.9%)
Caucasian	776 (54.0%)
Native Hawaiian/Pacific Islander	2 (0.1%)
Other	15 (1.1%)
Declined to answer	2 (0.1%)

Prior to initiation of the study, the clinical study protocol and the informed consent document were reviewed and approved by an Institutional Review Board (IRB) (RCRC Independent Review Board, Austin, TX), operating in accordance with Title 21 of the Code of Federal Regulations (CFR), Part 56. Three geographically distinct US study sites representing diverse POC settings (primary care medical office specializing in HIV care, Ft. Lauderdale, FL; HIV/AIDS community service organization, Phoenix, AZ; HIV testing outreach program, Philadelphia, PA), and one central laboratory (ACM Central Laboratory, Rochester, NY) were utilized. This was a double blinded, controlled study to evaluate the ability of untrained operators to correctly perform and interpret the results of the INSTI™ HIV-1 Antibody Test. The test was conducted by multiple untrained operators at the selected study sites using only the manufacturer's written instructions provided with the INSTI test kits. These operators had no prior experience with INSTI, received no training on the INSTI procedure, and did not complete any competency assessment panels prior to initiating the study. Table 2 lists the untrained operator demographics. The study participants were either high risk subjects with unknown HIV status who had consented to be tested for HIV at the time of study recruitment, or were known HIV-1 positive subjects recalled to the clinic for the study. For each study subject, venous blood collection and testing of fingerstick whole blood with the INSTI™ HIV-1 Antibody Test occurred on a single study day. Subjects were recruited between September 14, 2011 and January 4, 2012. Subjects were enrolled who were: (i) either at high risk for HIV infection with unknown HIV status, or known HIV-1 positive subjects willing to be re-tested in a blinded manner and had documentation of HIV-1 infection; (ii) willing to participate in the study site's standard of care HIV counseling and testing program and receive the study site's standard of care test

Table 2
Untrained operator demographics.

Operator ID	Site description	Experience	Education	Use rapid tests?[a]	Age
Site 1-001	Outreach	3 months	BA/BS	Yes	47
Site 1-002	Outreach	3.5 years	Some college	Yes	30
Site 1-005	Outreach	1 year	Some college	Yes	33
Site 1-006	Outreach	10 years	High school	Yes	44
Site 2-003	Dr. Office	1 year, 7 months	Tech school	Yes	47
Site 2-005	Dr. Office	7 years	Ph.D.	Yes	63
Site 2-006	Dr. Office	1 year, 9 months	Some college	Yes	27
Site 3-001	CSO[b]	Unknown	Unknown	Yes	Unknown
Site 3-004	CSO	Unknown	BA/BS	Yes	28
Site 3-008	CSO	12 years	Tech school	Yes	66
Site 3-009	CSO	Unknown	Unknown	Yes	44

Note: information on operator job title within the sites was not collected.
[a] Can be any rapid test, not including INSTI.
[b] Community Service Organization.

Table 3
Measures of agreement of INSTI with CM and respective 95% confidence intervals, $n = 1388$.

Measure of agreement	Point estimate	95% one sided CI[a]	95% 2 sided CI[a]
Percent positive agreement	517/517 (100%)	99.48–100	99.26–100
Percent negative agreement	869/871 (99.77%)	99.31–99.92	99.17–99.94

[a] Score method.

results; (iii) male or female subjects at least 18 years old; and (iv) willing to provide proper informed consent and the necessary volume of venous whole blood and finger stick blood for use in the study protocol testing methods. Subjects were excluded if they: (i) had participated previously in this study; (ii) self-reported a history of multiple myeloma (listed as a limitation in the INSTI package insert); (iii) were employees or immediate family members of bioLytical Laboratories or the study sites; (iv) were known HIV positive and self-reported a history of long-term anti-retroviral therapy of greater than ten (10) years. Subjects were considered high risk for HIV infection if any of the following were self-reported: Men who have/had sex with men; born to an HIV positive mother; had sex with an HIV positive partner; ever injected illegal drugs; had sex with multiple partners (defined as two or more within the preceding three months); had a current or prior history of sexually transmitted disease.

A total of 1388 subjects met the eligibility criteria and participated in the study of which 905 were high risk subjects with unknown HIV status and 483 subjects were known HIV positives. Recruitment rates across the three study sites varied: Site 1 enrolled 111 subjects with unknown HIV status and 33 known HIV positives; Site 2 enrolled 473 subjects with unknown HIV status and 303 known HIV positives; site 3 enrolled 321 subjects with unknown HIV status and 147 known HIV positives. For subjects with unknown HIV status a venous blood sample was obtained for testing at a central laboratory (ACM Global, Rochester, NY) by the ADVIA Centaur HIV-1/2/O Assay (CM), Siemens, Malvern, PA, and HIV-1 Western Blot Bio-Rad, Hercules, CA if necessary. From the same subject, a 50 μL fingerstick sample was obtained for performance of INSTI by an untrained operator at the study site. Results of INSTI obtained by untrained operators were compared to the results of the CM and WB performed by laboratory professionals. Enrollment of subjects with unknown HIV status across the three sites continued until a minimum of 30 *de novo* confirmed HIV positives were identified. For known HIV-1 positive subjects a 50 μL fingerstick sample was obtained for performance of INSTI by an untrained operator at the study site. All results for INSTI and Comparator Methods were interpreted according to the manufacturer's package insert instructions. INSTI results were compared to the subject HIV status determined from the site source documentation of the results of standard of care testing. All operators were blinded to subject identity and HIV status. The results of INSTI testing were not provided to the study subjects. The positive percent agreement (PPA) and negative percent agreement (NPA) between INSTI results obtained by untrained operators and CM results obtained by laboratory professionals were calculated along with 95% confidence intervals (Score method).

Additionally, a study was conducted to evaluate the ability of untrained operators using INSTI to detect HIV antibodies in weakly reactive samples. Randomly coded panels consisting of 4 contrived weakly reactive plasma samples were tested with INSTI at the study sites by 10 untrained operators (60 measurements per sample, 240 total INSTI tests: 10 operators testing 4 samples 6 times each over 5 days). The weakly reactive plasma samples were prepared by the study sponsor (bioLytical Laboratories, Inc., Richmond, BC, Canada) through serial dilution of known HIV positive plasma: a single HIV-1 positive plasma control was serially diluted with HIV negative plasma to represent INSTI HIV-1 results that were at the reactive endpoint (1 sample with an equal distribution of reactive and non-reactive INSTI HIV-1 Antibody Test results), slightly above endpoint (2 samples with a greater number of INSTI HIV-1 Antibody Test reactive results than non-reactive results) and slightly below endpoint (1 sample with a greater number of INSTI HIV-1 Antibody Test non-reactive results than reactive results).

Testing by untrained operators was conducted over a 5 day interval during the study period at the 3 study sites, with panel samples integrated with testing of study subject samples. The same panels were also tested by trained laboratory professionals at a single location to provide a basis for comparison of the distribution of results between untrained operators and the trained laboratory professionals.

4. Results

A total of 1418 subjects met eligibility criteria, from which 30 discontinued due to inability to obtain sufficient test samples, protocol violations, or failure to provide consent. The study subject demographics are described in Table 1. Of the 1388 subjects that completed the study, the positive percent agreement (PPA) and the negative percent agreement (NPA) between INSTI results obtained by untrained operators with fingerstick blood were compared to those of the CM results obtained by trained laboratory professionals on matching plasma from venous blood for HIV status unknown subjects. For the known HIV positives, the PPA and NPA of INSTI results were compared to the source-documented confirmed HIV antibody positive status. The results for the 1388 subjects are summarized in Table 3. For each calculation of the percent agreement, the 95% one-sided and two sided confidence intervals (Score method) are provided, revealing a high degree of agreement between INSTI results and the subjects' HIV status: PPA was 100% (517/517) and NPA was 99.8% (869/871). A total of two (2) INSTI false reactive results were interpreted by untrained operators, compared to the CM results. No further testing was conducted to resolve these discordant results. There were no INSTI invalid results obtained by any intended user for the study population.

Table 4 summarizes the data for the prospective, HIV status-unknown subjects in the study population. There were 34 previously undiagnosed HIV infections identified by INSTI and the CM in the study from the total of 905 prospective subjects who provided fingerstick blood and venous whole blood. There were no INSTI invalid results obtained by any intended user for this portion of the study population.

The results for the LoD study are presented in Table 5. For each panel specimen ($n = 4$), the percent of INSTI reactive results for the total tests performed ($n = 60$) by the 10 participating untrained operators was determined. The outcome was compared to the

Table 4
Results of INSTI compared to CM for HIV status unknown subjects ($n = 905$).

Insti result	Subject HIV status (CM)		
	Positive	Negative	Total
Positive	34[a]	2	36
Negative	0	869	869
Total	34	871	905

[a] Confirmed by FDA approved Western Blot Assay.

Table 5
Performance of the INSTI™ HIV-1 Antibody Test run by untrained operators with weakly reactive samples.

Sample	Dilution	Intended users	
		Percent reactive	95% Confidence interval
Weakly Reactive 1	1:600	88.3%[b] (53/60)	77.8–94.2%
Weakly Reactive 2	1:800	80.0% (48/60)	68.2–88.2%
Weakly Reactive 3	1:1200	66.1%[b] (39/59)[a]	53.4–76.9%
Weakly Reactive 4	1:1600	34.5% (20/58)[a]	23.6– 47.3%

[a] A total of 3 INSTI HIV-1 Antibody Test invalid results were obtained: 1 invalid for Weakly Reactive 3 sample and 2 invalids for Weakly Reactive 4 sample.
[b] Two out of 10 intended users had a lower number of reactive results with weakly reactive samples, as compared with other intended users.

expected results that were determined through an equal number of tests ($n = 60$) for each panel specimen conducted by trained laboratory professionals.

5. Conclusions and discussion

Untrained operators with varied background and no prior experience with INSTI, from clinical and non-clinical settings, were able to collect fingerstick blood and perform the test correctly by following only the instructions provided in the INSTI Package Insert and Quick Reference Guide, without any additional training. Their results were highly concordant with FDA approved laboratory based comparator method results obtained by trained professionals. There were no false negative results obtained by the untrained operators for the 517 HIV-1 antibody positive fingerstick blood samples collected from subjects tested in this study, for a positive percent agreement (equivalent to sensitivity) of 100% (95% CI 99.3–100%). The same operators interpreted a total of two (2) false positives from the 871 study subjects with HIV Negative status, for a negative percent agreement (equivalent to specificity) of 99.8% (95% CI 99.2–99.9%).

In comparison, this data is also highly concordant with data from the US device trials to determine safety and effectiveness of INSTI for the FDA Premarket Approval (granted in November, 2010) that was conducted by trained operators in monitored clinical trial settings (Clinical Trials.gov, NCT00514605). In that study, which used the same CM, overall INSTI sensitivity for fingerstick blood was 99.8% (1095/1097 HIV positives correctly identified, 95% CI = 99.3–99.9%), and specificity was 99.5% (1375/1382 HIV negatives correctly identified, 95% CI = 99.0–99.8%). This data is presented in the manufacturer's package insert.

This study clearly demonstrates that the 60 second INSTI HIV-1 Antibody Test can be performed on actual patient fingerstick blood samples by untrained operators in varied point of care settings without significant risk of erroneous results. Patients who present for HIV testing at point of care settings, especially in regions with high incidence, can have very low levels of detectable HIV antibodies in their blood samples at the time of testing. These low antibody levels often appear as very faint visual signals in rapid test methods that could be miss-interpreted as non-reactive by inexperienced test providers [17]. Correct interpretation of these faint reactions can be further influenced by lighting conditions and individual visual acuity of the test provider. Furthermore, it has been suggested by others that rapid tests combined with nucleic acid testing methods can maximize early detection of HIV in highly incident populations [18]. It was therefore important to demonstrate in the LoD portion of the study that a cross section of untrained operators was able to correctly interpret the results of specimens containing very low levels of HIV antibodies. These operators were able to meet the expected results as demonstrated in Table 5 for contrived plasma samples that contained very low levels of HIV antibody. This is an important demonstration that the insignificant risk of erroneous results can also apply to low levels of antibody.

In conclusion, as shown in this study, the INSTI HIV-1 Antibody Test employs a methodology that is so simple and accurate as to render the likelihood of erroneous results by untrained intended use operators in POC test settings negligible. After review of data from this study, CLIA waived status for INSTI was granted for fingerstick blood by FDA-CDRH in July, 2012.

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Competing interests

Authors Richard Galli, Amelia Daly and Christopher Shackleton are paid employees of bioLytical Laboratories. Author Kevin Green is a paid consultant of bioLytical Laboratories.

Ethical approval

This study was approved by the RCRC Independent Review Board, Austin, Texas, and was compliant with the principles of the Declaration of Helsinki: Recommendations guiding physicians in biomedical research involving human subjects. All subjects provided informed consent to participate in the study.

References

[1] Marks G, Crepaz N, Janssen RS. Estimating sexual transmission of HIV from persons aware and unaware that they are infected with the virus in the USA. AIDS LWW 2006;20(10):1447–50.
[2] The right to know: new approaches to HIV testing and counseling. Geneva: WHO; 2003.
[3] Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med 2011;365(August (6)):493–505.
[4] Montaner JSG, Lima VD, Barrios R, Yip B, Wood E, Kerr T, et al. Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. Lancet 2010;376(August (9740)):532–9.
[5] Bayer R, Oppenheimer GM. Routine HIV testing, public health, and the USPSTF—an end to the debate. N Engl J Med 2013;368(10):81–4. Mar 7;.
[6] Branson BM, Handsfield HH, Lampe MA, Janssen RS. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. MMWR 2006;55(RR14):1–17.
[7] World Health Organization. Revised recommendations for the selection and use of HIV antibody tests. UNAIDS, editor. Wkly Epidemiol Rec 1997;72(12):81–7.
[8] UNAIDS JUNPOHA, editor. The importance of simple/rapid assays in HIV testing. Wkly Epidemiol Rec 1998;73(October (42)):321–6.
[9] Branson BM. State of the art for diagnosis of HIV infection. Clin Infect Dis 2007;45(December (Suppl. 4)):S221–5.
[10] Everett DB, Baisley K, Changalucha J, Vallely A, Watson-Jones D, Cook C, et al. Suitability of simple human immunodeficiency virus rapid tests in clinical trials in community-based clinic settings. J Clin Microbiol 2009;47(April (4)):1058–62.
[11] Beckwith CG, Flanigan TP, del Rio C. It is time to implement routine, not risk-based, HIV testing. Clin Infect Dis 2005.
[12] Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 2010.
[13] St Lawrence JS, Montaño DE, Kasprzyk D, Phillips WR, Armstrong K, Leichliter JS. STD screening, testing, case reporting, and clinical and partner notification practices: a national survey of US physicians. Am J Public Health 2002;92(November (11)):1784–8.
[14] Pai NP, Sharma J, Shivkumar S, Pillay S, Vadnais C, Joseph L, et al. Supervised and unsupervised self-testing for HIV in high- and low-risk populations: a systematic review. PLoS Med 2013;10(April (4)):e1001414.
[15] FDA guidance for industry and FDA staff: recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) waiver applications for manufacturers of in vitro diagnostic devices [Internet]. Available from: http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm070890.pdf

[16] PEPFAR, WHO, USAID, CDC, LSTM, editors. Planning, implementing and monitoring home-based HIV testing and counselling. World Health Organization; 2012 September. p. 1–64.
[17] Learmonth KM, McPhee DA, Jardine DK, Walker SK, Aye T-T, Dax EM. Assessing proficiency of interpretation of rapid human immunodeficiency virus assays in nonlaboratory settings: ensuring quality of testing. J Clin Microbiol 2008;46(May (5)):1692–7.
[18] Stekler JD, Swenson PD, Coombs RW, Dragavon J, Thomas KK, Brennan CA, et al. HIV testing in a high-incidence population: is antibody testing alone good enough? Clin Infect Dis 2009;49(August (3)):444–53.