Journal of Medical Virology 80:484–493 (2008)

Evaluation of a New, Fully Automated Immunoassay for Detection of HTLV-I and HTLV-II Antibodies

Xiaoxing Qiu,[1]* Steven Hodges,[1] Teresa Lukaszewska,[1] Shigeo Hino,[2] Hiroyasu Arai,[3] Julie Yamaguchi,[1] Priscilla Swanson,[1] Gerald Schochetman,[1] and Sushil G. Devare[1]

[1]*Abbott Diagnostics, AIDS Research and Retrovirus Discovery, Abbott Park, Illinois*
[2]*Department of Virology, Tottori University, Yonago, Japan*
[3]*Abbott Japan, Tokyo, Japan*

Screening blood donations for human T-lymphotropic virus types I and II (HTLV-I/II) continues to be important in protecting the safety of blood products and controlling the global spread of these retroviruses. We have developed a fully automated, third generation chemiluminescent immunoassay, ARCHITECT rHTLV-I/II, for detection of antibodies to HTLV-I/II. The assay utilizes recombinant proteins and synthetic peptides and is configured in a double antigen sandwich assay format. Specificity of the assay was 99.98% (9,254/9,256, 95% CI = 99.92–100%) with the negative specimens from the general population including blood donors, hospital patients and pregnant women from the US, Japan and Nicaragua. The assay demonstrated 100% sensitivity by detecting 498 specimens from individuals infected with HTLV-I (n = 385) and HTLV-II (n = 113). ARCHITECT rHTLV-I/II results were in complete agreement with the Murex HTLV-I/II reference assay and 99.7% agreement with the Genelabs HTLV Blot 2.4 confirmatory assay. Analytical sensitivity of the assay was equivalent to Murex HTLV-I/II assay based on end point dilutions. Furthermore, using a panel of 397 specimens from Japan, the ARCHITECT rHTLV-I/II assay exhibited distinct discrimination between the antibody negative (Delta Value = −7.6) and positive (Delta Value = 7.6) populations. Based on the excellent specificity and sensitivity, the new ARCHITECT rHTLV-I/II assay should be an effective test for the diagnosis of HTLV-I/II infection and also for blood donor screening. ***J. Med. Virol. 80:484–493, 2008.***

KEY WORDS: HTLV-I; HTLV-II; serological assay; recombinant antigen; blood donor screening

INTRODUCTION

Human T-lymphotropic virus types I and II (HTLV-I/II) are closely related human retroviruses that were discovered in the early 1980s [Poiesz et al., 1980; Kalyanaraman et al., 1982; Yoshida et al., 1982]. HTLV-I causes adult T-cell leukemia (ATL) [Hinuma et al., 1981; Miyoshi et al., 1981; Blattner et al., 1983; Yamaguchi et al., 1984], HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) [Gessain et al., 1985; Rodgers-Johnson et al., 1985; Osame et al., 1986] and is associated with several other inflammatory disorders including uveitis [Watanabe et al., 1997], infective dermatitis [LaGrenade et al., 1990], polymyositis [Morgan et al., 1989], and HTLV-I associated arthritis [Nishioka et al., 1989; Yakova et al., 2005]. Endemic HTLV-I infection has been identified in southern Japan, the Caribbean basin, Central Africa, South America, and Melanesia [Proietti et al., 2005]. It is estimated that ~20 million individuals worldwide are infected with HTLV-I [Taylor et al., 2005]. Although the related retrovirus HTLV-II is less pathogenic than HTLV-I, it has been associated with a neurological disease similar to HAM/TSP [Araujo and Hall, 2004] and with chronic inflammatory arthropathy [Hjelle et al., 1992]. A high prevalence of HTLV-II infections has been found among intravenous drug users and some native inhabitants in the Americas and Africa [Lee et al., 1989; Gessain et al., 1995; Hall et al., 1996].

Three major transmission modes of HTLV-I/II have been well established: (1) sexual contact, (2) exposure to infected cellular blood products through blood transfusion or sharing injection needles, and (3) mother to child transmission through prolonged breast-feeding [Hino et al., 1985; Hall et al., 1996; Manns et al., 1999]. To prevent transfusion of HTLV-I/II infected blood, donor screening for the presence of HTLV-I/II antibodies was introduced in Japan in 1986, the United States in 1988, Canada in 1989, France in 1991, and several European and South American countries after 1991.

*Correspondence to: Xiaoxing Qiu, Abbott Diagnostics, D-09NG, Bldg. AP20, 100 Abbott Park Road, Abbott Park, IL 60064-6015. E-mail: xiaoxing.qiu@abbott.com

Accepted 30 October 2007
DOI 10.1002/jmv.21083
Published online in Wiley InterScience (www.interscience.wiley.com)

Since the implementation of donor screening, a decline in HTLV-I/II prevalence among blood donors has been observed both in Japan and the United States [Inaba et al., 1999; Dodd et al., 2002]. As observed for blood donations, prenatal screening for HTLV-I/II has also reduced mother to child transmission of HTLV-I/II infection in HTLV endemic regions, [Hino et al., 1994], and is being considered even in non-endemic areas [Proietti et al., 2005; Taylor et al., 2005; Alarcón et al., 2006].

Since the introduction of the first HTLV-I/II assays in the mid-1980s, considerable progress has been made over the past 20 years in the serologic detection of HTLV-I/II infection. Using terminology similar to that described for the progress made in HIV serological assays [Ly et al., 2001], evolution of HTLV-I/II assays can be defined as three generations. First generation HTLV assays utilized HTLV-I viral lysate proteins as the antigen source. Consequently, some HTLV-II infections were not identified by these assays [Andersson et al., 1999]. Second generation assays improved HTLV-II detection by including HTLV-II specific viral lysate or recombinant proteins. Most 1st and 2nd generation assays use an indirect assay format: anti-HTLV-I/II antibodies are captured by immobilized viral proteins and subsequently detected using polyclonal antibody conjugates to human immunoglobulins (Ig). Third-generation assays use a double antigen sandwich format: recombinant proteins and/or synthetic peptides are used to both capture and directly detect anti-HTLV-I/II antibodies. This assay configuration allows for efficient detection of IgG as well as the IgM antibodies to HTLV-I/II that are present during seroconversion [Gallarda et al., 1992; Burgisser et al., 1996]. Several comparative evaluations have demonstrated improved performance of 3rd generation assays relative to 1st and 2nd generation formats [Andersson et al., 1999, 2001; Vrielink et al., 1999]. Currently two microtiter based Enzyme-linked Immunoassays (EIA) in 3rd generation configuration are commercially available, Murex HTLV-I/II (GE80/81) and Ortho HTLV-I/HTLV-II Ab-Capture.

This study describes a fully automated 3rd generation HTLV-I/II serologic assay designed for the Abbott ARCHITECT® instrument. The ARCHITECT rHTLV-I/II assay uses HTLV-I and HTLV-II recombinant gp21 proteins and synthetic gp46 peptides in a double antigen sandwich assay format. Sensitivity and specificity of the assay were evaluated with confirmed HTLV-I and HTLV-II specimens and general populations from the US, Japan and Nicaragua.

MATERIALS AND METHODS

ARCHITECT rHTLV-I/II Assay

The ARCHITECT rHTLV-I/II assay combines paramagnetic microparticle and chemiluminescent immunoassay technologies in a high-throughput (200 tests per hour), fully automated, random-access ARCHITECT instrument system. The two-step immunoassay utilizes a double antigen sandwich assay format as illustrated in Figure 1. The first step combines sample (serum or plasma), assay diluent and paramagnetic microparticles. HTLV-I/II antibodies present in the sample are captured on paramagnetic microparticles coated with HTLV-I/II gp46 synthetic peptides or HTLV-II gp21 recombinant protein. The microparticles are washed to remove unbound proteins. In the second step, HTLV-I/II antibodies captured by the microparticles are incubated with acridinium-labeled HTLV-I/II gp46 synthetic peptides and HTLV-I gp21 recombinant antigen. Following an additional wash cycle, alkaline hydrogen peroxide solution is added to release acridinium chemiluminescent signal. The intensity of the chemiluminescence, measured as relative light units (RLU), is proportional to the concentration of HTLV-I/II antibodies in the sample.

The ARCHITECT rHTLV-I/II assay cutoff (CO) value was determined based on the following calculation: CO = 0.25(Calibrator 1 Mean RLU). Assay results were reported as the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen. Specimens with S/CO values <1.00 were considered non-reactive; specimens

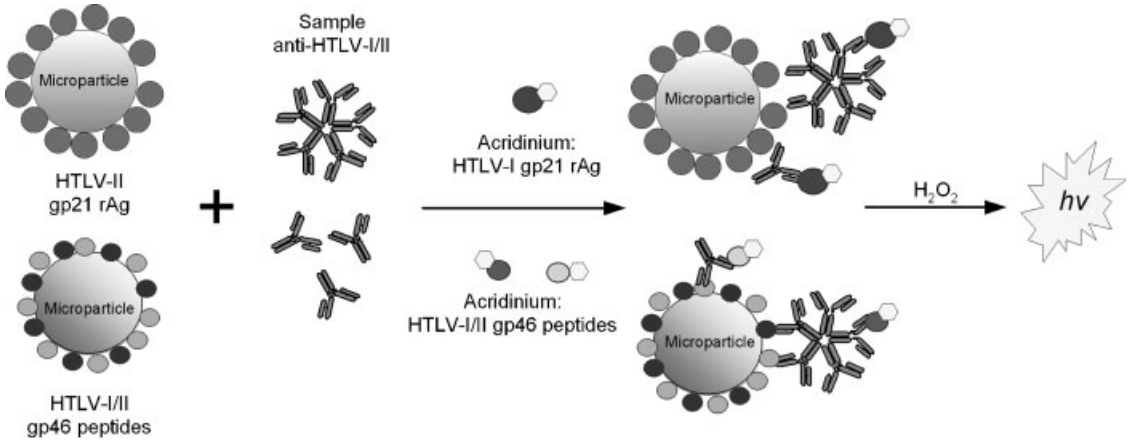

Fig. 1. Schematic diagram of the ARCHITECT rHTLV-I/II assay format. hv = chemiluminescent signal; gp21 rAg = recombinant gp21 antigen; Acridinium = Acridinium labeled conjugate.

with S/CO values ≥1.00 were considered initially reactive. Initially reactive specimens were retested in duplicate by the ARCHITECT rHTLV-I/II assay. Repeatedly reactive specimens were analyzed by HTLV-I/II reference and confirmatory assays described below. Anti-HTLV confirmed positive specimens were excluded from the specificity calculation.

Reference and Confirmatory Assays

Murex HTLV-I/II (GE80/81; Murex Diagnostics, Dartford, United Kingdom) was used as the reference assay to retest ARCHITECT rHTLV-I/II repeatedly reactive specimens and to evaluate serial dilutions of anti-HTLV-I/II positive specimens. PRISM® HTLV-I/HTLV-II (Abbott Laboratories, Abbott Park, IL) and SERODIA-HTLV-I (Fujirebio, Tokyo, Japan) assays were used for comparative evaluation of selected Japanese specimens. Confirmation of HTLV antibodies was performed by Western blot (WB) analysis using HTLV Blot 2.4 (Genelabs Diagnostics, Pte. Ltd., Redwood City, CA), and results were interpreted according to criteria defined by the manufacturer. Specimens reactive by both ARCHITECT rHTLV-I/II and Murex HTLV-I/II but negative or indeterminate by WB were further tested by radioimmunoprecipitation (RIPA) at Specialty Laboratories (Valencia, CA). All commercial assays were performed according to the instructions of the manufacturer.

Specimen Populations

Specificity was evaluated using a total of 9,276 specimens from the following populations:

(i) 2,608 random blood donor specimens (600 sera and 2,008 plasma) from the Gulf Coast Regional Blood Center, Houston, TX and supplied by ProMedDx LLC, Norton, MA;
(ii) 410 random blood donor specimens (fresh sera) from Nicaragua and supplied by Teragenix, Fort Lauderdale, FL;
(iii) 2,157 diagnostic specimens from Massachusetts hospital patients (1,010 sera and 1,147 plasma) and supplied by ProMedDx LLC;
(iv) 2,000 diagnostic specimens from Sapporo Kosei General Hospital, Hokkaido, Japan;
(v) 2,000 prescreened SERODIA-HTLV-1 antibody negative specimens from Tokyo, Japan;
(vi) 101 specimens (plasma and sera) from pregnant women in New York and Massachusetts including 1st trimester (50), 2nd trimester (44), 3rd trimester (7) and supplied by ProMedDx, LLC.

All specimens were collected according to the relevant guidelines and or institutional policies of each country or vendor.

Sensitivity was determined using the following panels:

Panel A: 181 HTLV antibody positive US blood donor specimens (sera and plasma) confirmed by WB/RIPA/PCR (75 HTLV-I, 106 HTLV-II) [Lee et al., 1991].

Panel B: 397 samples identified as HTLV-I antibody positive by SERODIA-ATLA-I in 1988 and obtained from Nagasaki University, Japan.

Panel C: Anti-HTLV I/II Mixed Titer Performance Panel PRP206 (Boston Biomedica Inc., Bridgewater, MA) including 14 HTLV confirmed positive samples (7 HTLV-I, 7 HTLV-II) and 1 seronegative control.

Panel D: Dilution series from three HTLV-I and three HTLV-II antibody positive specimens selected from Panel A. Twofold serial dilutions were prepared using normal human plasma negative for HBsAg, Syphilis, HIV antigen and antibodies to HCV, HIV-1/2, and HTLV-I/II.

Statistical Analysis

The 95% confidence interval (CI) was calculated using the Bayesian method and the exact CI for a proportion. Statistical significance was calculated using Fisher's exact *t*-test. *P*-values <0.05 were considered to be statistically significant.

The Delta Value is defined as the distance between the population mean and the CO, expressed in standard deviation (SD) units derived from the $\log_{10}$ transformed population distribution [Crofts et al., 1988]. Log_{10} transformation of the population S/CO values produces a more normal distribution pattern, that is the mean and SD of the transformed population S/CO values meet the statistical assumption of a normal distribution.

$$\text{Delta Value}\,(\delta) = \frac{\text{Population Mean}\,(\log \text{S/CO})}{\text{SD}\,(\log \text{S/CO})}$$

RESULTS

Assay Specificity

The specificity of the ARCHITECT rHTLV-I/II assay was evaluated on blood donor specimens from the United States (US, n = 2,608) and Nicaragua (n = 410; Table I). The majority of specimens (3,016 of 3,018) had S/CO values <1.00 and were considered non-reactive. Two ARCHITECT rHTLV-I/II repeatedly reactive specimens were also reactive in the Murex HTLV-I/II assay and were confirmed positive by WB (Table II). The US donor was infected with HTLV-II and the Nicaraguan donor with HTLV-I. Consequently, these two samples were removed from the specificity calculation. Thus, on the 3,016 blood donor specimens, the specificity of ARCHITECT rHTLV-I/II was 100% (95% CI = 99.88–100%).

Of 2,157 diagnostic specimens from US hospital patients, three samples were repeatedly reactive by the ARCHITECT rHTLV-I/II assay (Table I). However, only one of these specimens was reactive in Murex HTLV-I/II and was confirmed as HTLV-II by WB (Table II). Thus, two specimens were considered false positive by the ARCHITECT rHTLV-I/II assay. Since the diagnostic specimens were unlinked, the

TABLE I. Specificity of ARCHITECT rHTLV-I/II Assay

Population	N	Initial reactive % (n)	Repeat reactive % (n)	Non-confirmed[a] repeat reactive % (n)	% Specificity
US random blood donors	2,608	0.03% (1)	0.03% (1)	0.00% (0)	100%
Nicaraguan random blood donors	410	0.24% (1)	0.24% (1)	0.00% (0)	100%
US diagnostic specimens	2,157	0.14% (3)	0.14% (3)[b]	0.09% (2)	99.91%
Japanese diagnostic specimens	2,000	0.85% (17)	0.85% (17)	Not Done	100%[c]
Prescreened negative Japanese specimens	2,000	0.00% (0)	0.00% (0)	0.00% (0)	100%
US pregnant women	101	0.10% (1)	0.00% (0)	0.00% (0)	100%
Total	9,276	0.25% (23)	0.24% (22)	0.02% (2)	99.98%

[a]Not confirmed HTLV positive by WB.
[b]Only one of three repeat reactive specimens was confirmed as HTLV-II positive by WB, leaving two non-confirmed repeat reactive specimens for calculation of specificity.
[c]Seventeen specimens were repeat reactive by Fujirebio SERODIA HTLV-1 PA but were not tested by WB due to depletion of sample volume.

disease state of the two false positive individuals is unknown. Excluding the HTLV-II confirmed specimen, the calculated assay specificity on a US diagnostic population was 99.91% (2,154/2,156, 95% CI = 99.67–99.99%; Table I). Specimens from Japan were also used to assess ARCHITECT rHTLV-I/II assay specificity. A high-risk hospital diagnostic population of 2,000 patients from Hokkaido, Japan had 17 repeatedly reactive specimens with S/COs ranging from 4 to 134 (Table I). All 17 specimens were also repeatedly reactive with SERODIA HTLV-I but could not be further confirmed by WB due to depletion of sample volume. Based on the assumption that the 17 repeatedly reactive specimens will be confirmed, the specificity of ARCHITECT rHTLV-I/II was 100% (1983/1983, 95% CI = 99.81–100%). No ARCHITECT rHTLV-I/II repeatedly reactive specimens were identified among 2,000 prescreened SERODIA HTLV-I non-reactive specimens from Tokyo.

To assess the suitability of the assay for prenatal screening, specimens from 101 pregnant women were evaluated by ARCHITECT rHTLV-I/II assay. One initial reactive specimen was non-reactive on repeat testing. Therefore, specificity of this population was 100% (Table I).

In summary, the overall specificity of the ARCHITECT rHTLV-I/II assay was 99.98% (9254/9256, 95% CI = 99.92–100%) on general populations from the US, Japan and Nicaragua (Table I). The similar initial reactive rate (0.25%) and repeat reactive rate (0.24%) demonstrated the robust performance of the assay.

The ARCHITECT rHTLV-I/II assay demonstrated narrow distributions of S/CO values for non-reactive specimens (S/CO <1.0) from the general populations (Fig. 2). Similar CO values were observed across all populations tested: 28 SD from the mean for the US blood donors, 17 SD for Nicaraguan blood donors, and 22 SD for the Japanese pre-screened negative population. US and Japanese diagnostic populations showed CO values of 21 and 29 SD from the mean, respectively. In general, assays with CO values greater than 10 SD from the population mean are considered to be suitable for screening [Shah et al., 2003; Sickinger et al., 2004].

Assay Sensitivity

The qualitative sensitivity of the ARCHITECT rHTLV-I/II assay was evaluated using confirmed HTLV antibody positive specimens. Distribution of S/CO values for an in-house collection of 181 samples from subjects with confirmed HTLV-I (n = 75) or HTLV-II (n = 106) infection is shown in Figure 3. In addition, ARCHITECT rHTLV-I/II reactivity was compared with three reference assays using an anti-HTLV I/II performance panel from Boston Biomedica, Inc. (BBI) including seven HTLV-I and seven HTLV-II members (Table III). The assay demonstrated 100% sensitivity by detecting all 181 of the in-house samples and 14 BBI panel members with S/CO values ranging from 10 to 180. One HTLV negative BBI panel member was below the CO of each of the four comparative assays.

The sensitivity of the ARCHITECT rHTLV-I/II assay was further evaluated with a 397-member panel obtained from Nagasaki University. The samples were identified as HTLV-I positive in 1988 using a 1st version Fujirebio SERODIA-ATLA Particle Agglutination

TABLE II. Confirmation of ARCHITECT rHTLV-I/II Repeatedly Reactive Specimens

Specimen	ARCHITECT rHTLV-I/II S/CO	MUREX HTLV-I + II S/CO	WB reactivity	Final interpretation
US donor	42.9	>10.8	GD21, p24, p36 rgp46-II	HTLV-II
Nicaraguan donor	148	>11.6	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I
US diagnostics-1	71.5	>11.8	GD21, p19, p24, p28, p36, gp46, p53, rgp46-II	HTLV-II
US diagnostics-2	1.8	0.62	No bands	Negative
US diagnostics-3	1.8	0.51	No bands	Negative

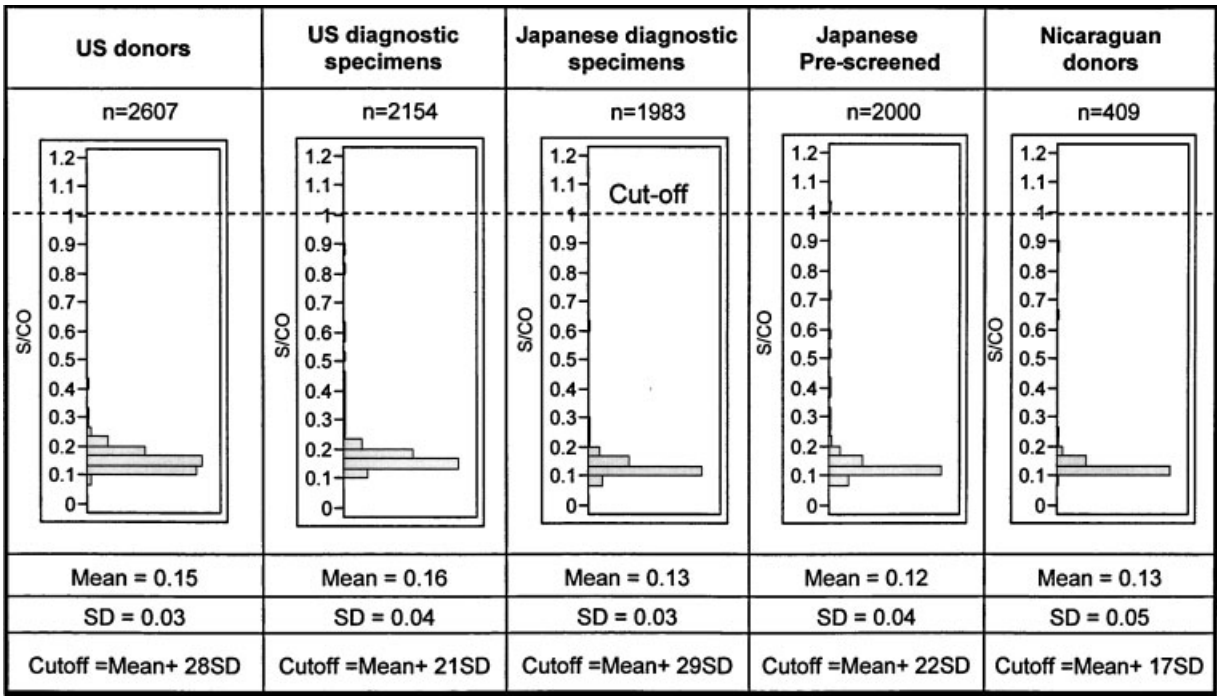

Fig. 2. Distribution of S/CO values for ARCHITECT rHTLV-I/II non-reactive populations.

assay. At the time, HTLV-I/II reactivity was not confirmed by WB, immunofluorescence or PCR. In this study, all the panel members were tested in parallel with three assays: (i) Murex HTLV-I/II, (ii) PRISM HTLV-I/II and (iii) ARCHITECT rHTLV-I/II. Of the 397 Japanese specimens, 303 (76%) were repeatedly reactive by all 3 screening assays. The ARCHITECT rHTLV-I/II S/CO values ranged from 4 to 215. All 303 repeatedly reactive specimens were confirmed anti-HTLV-I positive by WB (Table IV). A total of 92 specimens were categorized as non-reactive by all three assays: S/CO values ranged from 0.07 to 0.48 on the ARCHITECT rHTLV-I/II assay. Ten of these non-reactive specimens with S/CO values between 0.2 and 0.48 were selected for WB analysis: all 10 were negative for antibodies to HTLV-I/II (Table IV). Two of the 397 specimens showed discrepant serologic results (Table IV). One specimen was reactive on PRISM HTLV-I/II (S/CO = 3.0) but non-reactive on both the ARCHITECT rHTLV-I/II (S/CO = 0.09) and Murex HTLV-I/II (S/CO = 0.50) assays and was WB negative. Thus, this specimen was interpreted to be a true negative. The second specimen was repeatedly reactive on both the ARCHITECT rHTLV-I/II (S/CO = 1.3) and Murex HTLV-I/II (S/CO = 1.1) assays but was non-reactive on PRISM HTLV-I/II (S/CO = 0.8). This specimen was WB indeterminate (strong GD21 and weak p24 reactivity) and RIPA negative. Excluding this indeterminate specimen, the

TABLE III. Comparative Performance of the BBI Anti-HTLV I/II Mixed Titer Performance Panel

Panel ID	Matrix	HTLV type	Abbott HTLV-I/II EIA (S/CO)[a]	BioMerieux HTLV-I/II EIA (S/CO)[a]	Murex HTLV-I/II (S/CO)[a]	ARCHITECT rHTLV-I/II (S/CO)
PRP206-01	Plasma	I	>4.4	4.2	>11.7	63.8
PRP206-02	Plasma	II	>4.4	5.2	>11.7	62.2
PRP206-03	Plasma	II	>4.4	7.0	>11.7	45.2
PRP206-04	Plasma	I	>4.4	5.1	>11.7	20.8
PRP206-05	Plasma	II	>4.4	4.1	>11.7	40.9
PRP206-06	Serum	I	>4.4	4.5	>11.7	29.2
PRP206-07	Plasma	II	>4.4	4.3	>11.7	50.2
PRP206-08	Plasma	I	>4.4	7.1	>11.7	160.6
PRP206-09	Plasma	II	>4.4	5.2	>11.7	30.2
PRP206-10	Plasma	Negative	0.5	0.3	0.1	0.2
PRP206-11	Plasma	II	>4.4	3.3	>11.7	22.3
PRP206-12	Serum	I	>4.4	2.4	>11.7	50.0
PRP206-13	Serum	I	>4.4	7.2	>11.7	73.5
PRP206-14	Plasma	II	2.8	2.9	>11.7	70.6
PRP206-15	Serum	I	>4.4	6.2	>11.7	45.4

[a]S/CO values provided by BBI.

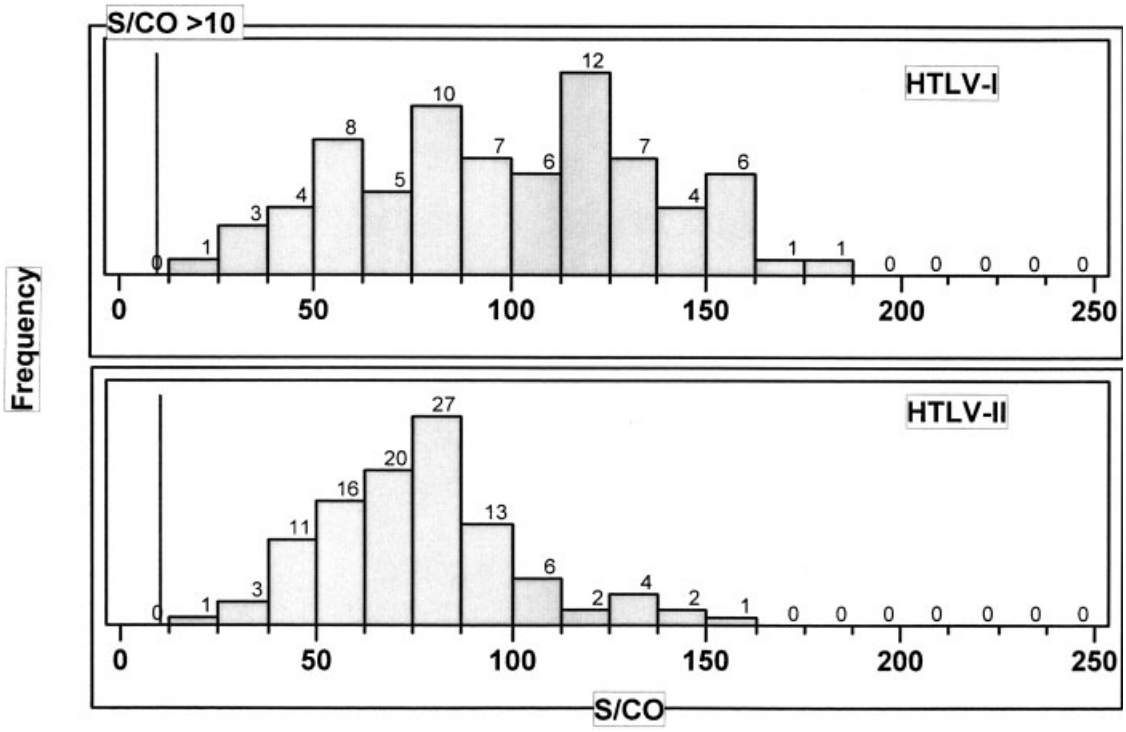

Fig. 3. Distribution of ARCHITECT rHTLV-I/II S/CO values for 181 HTLV positive specimens. Numbers of specimens within each S/CO value are shown above the solid bars.

sensitivity of the ARCHITECT rHTLV-I/II assay was 100% on the 303 HTLV-I positive Japanese specimens. Furthermore, WB confirmed 303 of 304 reactive specimens, resulting in 99.7% concordance between the ARCHITECT rHTLV-I/II assay and WB. ARCHITECT rHTLV-I/II and Murex HTLV-I/II assays were in complete concordance on the 397 Japanese specimens.

Since the Nagasaki panel (n = 397) contains both HTLV antibody negative and positive specimens, it is ideal for analyzing the separation between the two populations. The Delta (δ) value provides a mathematical method to quantify the degree of separation between positive and negative populations and to correspondingly determine the adequacy of the defined assay CO. A higher absolute value of δ correlates with a higher probability that the assay will correctly identify specimens as either antibody positive or negative [Crofts et al., 1988; Maskill et al., 1988]. The ARCHITECT rHTLV-I/II S/CO values of the Nagasaki panel members were $\log_{10}$ transformed and then the δ values were calculated. As demonstrated in Figure 4, the $\log_{10}$ S/CO distribution shows a clear discrimination of positive and negative populations with δ values of 7.6 below and above the CO. A similar calculation performed on the Murex and PRISM HTLV-I/II S/CO values gave the absolute δ values of 6.0 and 4.1, respectively.

Analytical Sensitivity

Because HTLV-I and HTLV-II seroconversion series are very rare and unavailable [Vrielink et al., 1999; Andersson et al., 2001], the analytical sensitivity of the ARCHITECT rHTLV-I/II assay was assessed using serial dilutions of six anti-HTLV confirmed specimens (three HTLV-I, three HTLV-II). Assay sensitivity was determined as the end-point dilution with a S/CO value of >1.00. The dilution series were tested in ARCHITECT rHTLV-I/II and Murex HTLV-I/II (Table V). Both assays showed equivalent end-point dilutions on five of six dilution series: three HTLV-I and two HTLV-II specimens. Murex HTLV-I/II was twofold more sensitive on one HTLV-II specimen. Overall, analytical sensitivity of the ARCHITECT rHTLV-I/II was equivalent to the Murex HTLV-I/II assay.

TABLE IV. Summary of Testing Results on the Nagasaki Panel (n = 397)

N	ARCHITECT rHTLV-I/II	Murex HTLV-I/II	PRISM HTLV-I/II	WB confirmation	Final interpretation
303	+	+	+	+	HTLV-I
1	+	+	−	Indeterminate	HTLV indeterminate
1	−	−	+	−	Negative
10	−	−	−	−	Negative
82	−	−	−	Not done	Negative

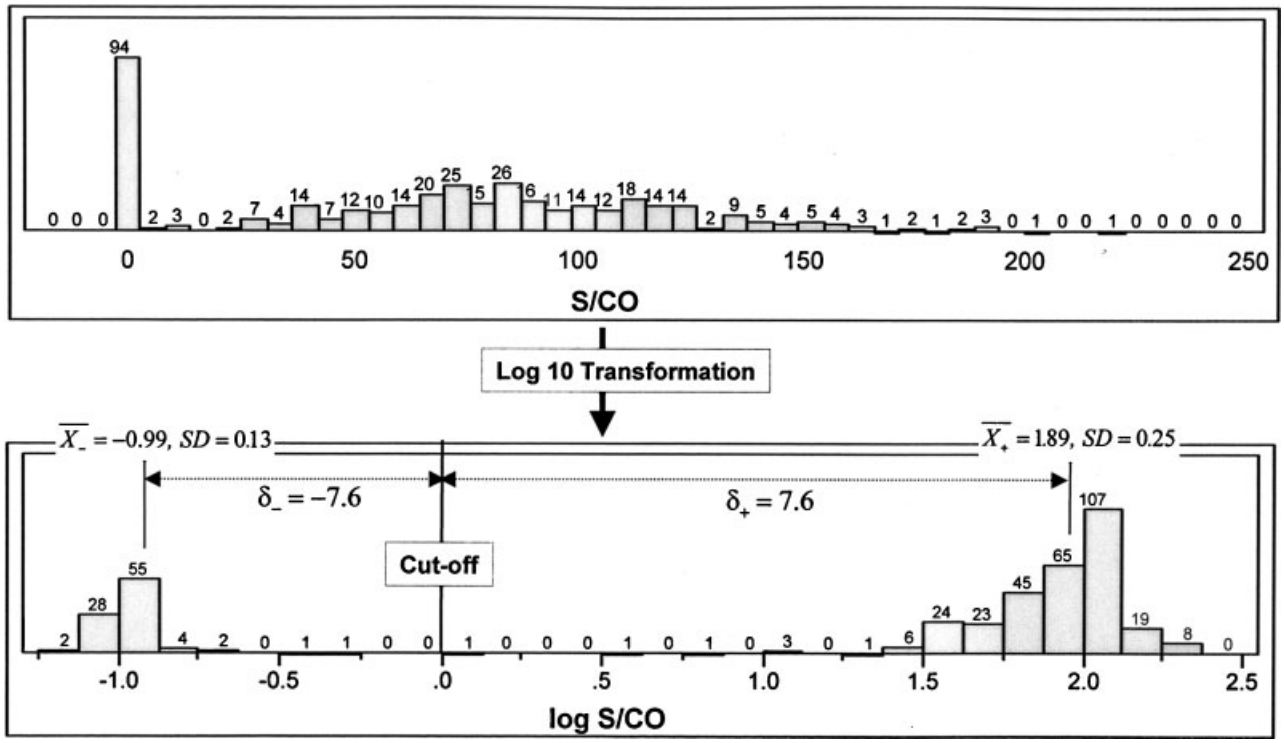

Fig. 4. Distribution of ARCHITECT rHTLV-I/II assay S/CO and $\log_{10}$ S/CO values on the Nagasaki panel (n = 397). Numbers of specimens within each S/CO or Log_{10} S/CO value are shown above the solid bars.

DISCUSSION

HTLV-I and HTLV-II infections are typically diagnosed using screening assays that detect the presence of virus-specific antibodies. Specificity of newer 3rd generation assays has been improved by including recombinant proteins and peptide antigens in a double antigen sandwich assay format. In comparative evaluations of 14 anti-HTLV serologic assays, the 3rd generation format Murex HTLV-I/II (GE80/81) assay exhibited

TABLE V. Analytical Sensitivity Comparison on Anti-HTLV Dilution Series

Specimen type	Dilution	ARCHITECT rHTLV-I/II (S/CO)	Murex HTLV-I + II (S/CO)
HTLV-I	1:1,000	2.41	1.70
	1:2,000	1.26	1.12
	1:4,000	0.72	0.76
	1:8,000	0.43	0.67
HTLV-I	1:20	2.53	2.02
	1:40	1.42	1.20
	1:80	0.75	0.74
HTLV-I	1:1,000	4.48	3.10
	1:2,000	2.18	1.87
	1:4,000	1.19	1.18
	1:8,000	0.70	0.74
	1:16,000	0.41	0.61
HTLV-II	1:50	2.84	2.49
	1:100	1.54	1.43
	1:2,000	0.82	0.96
	1:4,000	0.44	0.71
HTLV-II	1:200	2.48	2.51
	1:400	1.45	1.64
	1:800	0.75	1.07
	1:1,600	0.44	0.76
HTLV-II	1:400	2.42	2.30
	1:800	1.35	1.45
	1:1,600	0.73	0.98
	1:3,200	0.45	0.77

The last detectable dilutions (end point dilutions) are underlined.

the best-in-class performance [Andersson et al., 1999, 2001; Vrielink et al., 1999].

In this study, the performance of the recently developed, fully automated ARCHITECT rHTLV-I/II chemiluminescent immunoassay was evaluated. The assay incorporates recombinant proteins and synthetic peptides derived from the highly immunogenic transmembrane (gp21) and envelope (gp46) proteins of HTLV-I/II [Palker et al., 1989; Horal et al., 1991; Hadlock et al., 1995; Varma et al., 1995]. More importantly, the HTLV-I and HTLV-II recombinant gp21 antigens are configured in a double antigen sandwich format (Fig. 1).

Crystal structure analysis reveals that the HTLV-I gp21 ectodomain exhibits a trimeric helical hairpin structure similar to the ectodomain of HIV-1 [Kobe et al., 1999]. The immunodominant region of gp21 (aa 374–400) is partially comprised of an N-terminal trimeric coiled-coil and an adjacent disulfide-bonded loop, forming multiple complex conformational epitopes. Sequence analysis (data not shown) indicates that the immunodominant region of gp21 is 100% conserved between HTLV-I and HTLV-II as well as the recently discovered HTLV-III [Calattini et al., 2006; Switzer et al., 2006], whereas the remainder of the gp21 ectodomain is less conserved (~75% homology). Thus, the gp21 double antigen assay format of HTLV-II rgp21-coated microparticles and HTLV-I rgp21 acridinium-labeled conjugate not only preserves the highly conserved conformational epitopes of the gp21 immunodominant region but also minimizes non-specific binding known to be associated with the gp21 C-terminal region. [Hartley et al., 1991; Lal et al., 1992; Varma et al., 1995].

In contrast to the gp21 antigen, the immunodominant region of gp46 is less conserved between HTLV-I and HTLV-II with about 65% sequence homology. Thus, in the ARCHITECT rHTLV-I/II assay, the HTLV-I/II gp46 peptides were configured on both sides of the double antigen assay format. The presence of gp46 peptides in the assay enhances the detection of samples having immunoreactivity directed against gp46.

The ARCHITECT rHTLV-I/II assay demonstrated 99.98% specificity on populations of specimens from US, Japan and Nicaragua including blood donors, hospital patients and pregnant women and 100% sensitivity on 498 confirmed HTLV-I/II positive specimens from the US and Japan. In addition, the analytical sensitivity of the assay was equivalent to the most sensitive Murex HTLV-I/II (GE80/81) assay.

HTLV antibody negative and positive populations were clearly discriminated by the ARCHITECT rHTLV-I/II assay. Of 397 Japanese specimens identified as HTLV-I positive in 1988 by the 1st version Fujiribio SERODIA-ATLA, 93 were antibody negative in the ARCHITECT rHTLV-I/II test. SERODIA-ATLA launched in1986 was less specific, and had a false positive rate of ~26% as compared to the improved version of the SERODIA-HTLV-I [Yamada et al., 1992; Inaba et al., 1999]. In addition, a comparative evaluation showed that the improved SERODIA-HTLV-I had similar specificity and sensitivity as compared to both Murex HTLV-I/II and PRISM HTLV-I/II [Andersson et al., 1999]. Therefore, it is not surprising that ~23% (93/397) of 1st version SERODIA-ATLA positive specimens were negative by ARCHITECT rHTLV-I/II, Murex HTLV–I/II and PRISM HTLV-I/II assays.

Although it is beyond the scope of this article to estimate the prevalence of HTLV-I/II, results from this study provide a representation of HTLV-I/II prevalence estimates in the evaluated populations (Table VI). The prevalence rate of 0.038% in the US blood donor population from the Gulf Coast region appeared higher than the 0.0096% prevalence rate reported by the American Red Cross [Dodd et al., 2002]. However, due to the large CI in this population (95% CI = 0.0093–0.21), the difference is not significant. Furthermore, the donor specimens were from a geographic region known to have a higher HTLV-I/II prevalence versus the entire US.

One HTLV-II antibody positive specimen was identified from the Massachusetts area hospital patients. All of the 2,157 hospital specimens were anonymous (identity unlinked) and no demographic information was available. Nevertheless, it is clear that the estimated 0.046% prevalence rate of HTLV-II in the US diagnostic population was significantly ($P < 0.001$) lower than the 0.85% prevalence rate of HTLV-I in the Japanese diagnostic population. The 2,000 Japanese diagnostic specimens were collected from Sapporo Kosei General Hospital in the northern island Hokkaido, where the prevalence rate of HTLV-I in the blood donor population is 0.5–1.0% compared to the blood donor population in the endemic region of southwestern Japan (>4%) as reported in 1994 [Ministry of Health, Labor and Welfare of Japan website, www.mhlw.go.jp].

To our knowledge, the prevalence rate of HTLV-I/II is still unknown in Nicaragua. Because Nicaragua is in geographic proximity to the HTLV-endemic areas of Jamaica, Colombia, Brazil, Argentina, Peru and French Guyana, it is not surprising to observe a 0.24% prevalence rate in the Nicaraguan blood donor population, although the number of blood donors screened in this study was small (n = 410).

In conclusion, our limited prevalence data indicated that HTLV-I and HTLV-II are still circulating among

TABLE VI. Estimated HTLV-I/II Prevalence Rates

Population	No. of specimens	No. of confirmed HTLV	% HTLV positive	95% CI
US blood donors	2,608	1 HTLV-II	0.038	0.0093–0.21
US diagnostic	2,157	1 HTLV-II	0.046	0.0112–0.25
Nicaragua blood donor	410	1 HTLV-I	0.24	0.059–1.35
Japanese diagnostic	2,000	17 HTLV-I	0.85	0.534–1.356

general populations even in the low prevalence countries such as the United States. Therefore, it is important to continue blood screening for HTLV-I/II to control the further spread of these human retroviruses. Performance evaluation data from this study clearly demonstrated that the high throughput and fully automated ARCHITECT rHTLV-I/II chemiluminescent immunoassay should effectively serve this purpose.

REFERENCES

Alarcón JO, Friedman HB, Montano SM, Zunt JR, Holmes KK, Quinnan GV. 2006. High endemicity of human T-cell lymphotropic virus type 1 among pregnant women in Peru. J Acquir Immune Defic Syndr 42:604–609.

Andersson S, Thorstensson R, Ramirez KG, Krook A, von Sydow M, Dias F, Biberfeld G. 1999. Comparative evaluation of 14 immunoassays for detection of antibodies to the human T-lymphotropic virus types I and II using panels of sera from Sweden and West Africa. Transfusion 39:845–851.

Andersson S, Gessain A, Taylor GP. 2001. Pooling of samples for seroepidemiological surveillance of human T-cell lymphotropic virus types I and II. Virus Res 78:101–106.

Araujo A, Hall WW. 2004. Human T-lymphotropic virus type II and neurological disease. Ann Neurol 56:10–19.

Blattner WA, Takatsuki K, Gallo RC. 1983. Human T-cell lukemia-lymphoma virus and adult T-cell leukemia. JAMA 250:1074–1080.

Burgisser P, Simon F, Wernli M, Wust T, Beya MF, Frei PC. 1996. Multicenter evaluation of new double-antigen sandwich enzyme immunoassay for measurement of anti-human immunodeficiency virus type 1 and type 2 antibodies. J Clin Microbiol 34:634–637.

Calattini S, Chevalier SA, Duprez R, Afonso P, Froment A, Gessain A, Mahieux R. 2006. Human T-cell lymphotropic virus type 3: Complete nucleotide sequence and characterization of the human tax3 protein. J Virol 80:9876–9888.

Crofts N, Maskill W, Gust ID. 1988. Evaluation of enzyme-linked immunosorbent assays: A method of data analysis. J Virol Methods 22:51–59.

Dodd RY, Notari EP, Stramer SL. 2002. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 42:975–979.

Gallarda JL, Henrard DR, Liu D, Harrington S, Stramer SL, Valinsky JE, Wu P. 1992. Early detection of antibody to human immunodeficiency virus type 1 by using an antigen conjugate immunoassay correlates with the presence of immunoglobulin M antibody. J Clin Microbiol 30:2379–2384.

Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de Thé G. 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 2:407–410.

Gessain A, Mauclère P, Froment A, Biglione M, Le Hesran JY, Tekaia F, Millan J, de The G. 1995. Isolation and molecular characterization of a human T-cell lymphotropic virus type II (HTLV-II), subtype B, from a healthy Pygmy living in a remote area of Cameroon: An ancient origin of HTLV-II in Africa. Proc Natl Acad Sci USA 92:4041–4045.

Hadlock KG, Goh CJ, Bradshaw PA, Perkins S, Lo J, Kaplan JE, Khabbaz R, Foung SK. 1995. Delineation of an immunodominant and human T-cell lymphotropic virus (HTLV)-specific epitope within the HTLV-I transmembrane glycoprotein. Blood 86:1392–1399.

Hall WW, Ishak R, Zhu SW, Novoa P, Eiraku N, Takahashi H, Ferreira M, da C, Azevedo V, Ishak MO, Ferreira O, Monken C, Kurata T. 1996. Human T-lymphotropic virus type II (HTLV-II): Epidemiology, molecular properties and clincial features of infection. J Acquir Immune Defic Syndr Hum Retrovir 13:S204–S214.

Hartley TM, Malone GE, Khabbaz RF, Lal RB, Kaplan JE. 1991. Evaluation of a recombinant human T-cell lymphotropic virus type I (HTLV-I) p21E antibody detection enzyme immunoassay as a supplementary test in HTLV-I/II antibody testing algorithms. J Clin Microbiol 29:1125–1127.

Hino S, Yamaguchi K, Katamine S, Sugiyama H, Amagasaki T, Kinoshita K, Yoshida Y, Doi H, Tsuji Y, Miyamoto T. 1985. Mother-to-child transmission of human T-cell leukemia virus type-I. Jpn J Cancer Res 76:474–480.

Hino S, Katamine S, Kawase K, Miyamoto T, Doi H, Tsuji Y, Yamabe T. 1994. Intervention of maternal transmission of HTLV-1 in Nagasaki, Japan. Leukemia 8:S68–S71.

Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, Miyoshi I. 1981. Adult T-cell leukemia: Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci USA 78:6476–6480.

Hjelle B, Appenzeller O, Mills R, Alexander S, Torrez-Martinez N, Jahnke R, Ross G. 1992. Chronic neurodegenerative disease associated with HTLV-II infection. Lancet 339:645–646.

Horal P, Hall WW, Svennerholm B, Lycke J, Jeansson S, Rymo L, Kaplan MH, Vahlne A. 1991. Identification of type-specific linear epitopes in the glycoproteins gp46 and gp21 of human T-cell leukemia viruses type I and type II using synthetic peptides. Proc Natl Acad Sci USA 88:5754–5758.

Inaba S, Okochi K, Sato H, Fukada K, Kinukawa N, Nakata H, Kinjyo K, Fujii F, Maeda Y. 1999. Efficacy of donor screening for HTLV-I and the natural history of transfusion-transmitted infection. Transfusion 39:1104–1110.

Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. 1982. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571–573.

Kobe B, Center RJ, Kemp BE, Poumbourios P. 1999. Crystal structure of human T cell leukemia virus type 1 gp21 ectodomain crystallized as a maltose-binding protein chimera reveals structural evolution of retroviral transmembrane proteins. Proc Natl Acad Sci USA 96:4319–4324.

LaGrenade L, Hanchard B, Fletcher V, Cranston B, Blattner W. 1990. Infective dermatitis of Jamaican children: A marker for HTLV-I infection. Lancet 336:1345–1347.

Lal RB, Brodine S, Kazura J, Mbidde-Katonga E, Yanagihara R, Roberts C. 1992. Sensitivity and specificity of a recombinant transmembrane glycoprotein (rgp21)-spiked Western immunoblot for serological confirmation of human T-cell lymphotropic virus type I and type II infections. J Clin Microbiol 30:296–299.

Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen IS. 1989. High rate of HTLV-II infection in seropositive i.v. drug abusers in New Orleans. Science 244:471–475.

Lee HH, Swanson P, Rosenblatt JD, Chen IS, Sherwood WC, Smith DE, Tegtmeier GE, Fernando LP, Fang CT, Osame M, Kleinman SH. 1991. Relative prevalence and risk factors of HTLV-I and HTLV-II infection in US blood donors. Lancet 337:1435–1439.

Ly TD, Martin L, Daghfal D, Sandridge A, West D, Bristow R, Chalouas L, Qiu X, Lou SC, Hunt JC, Schochetman G, Devare SG. 2001. Seven human immunodeficiency virus (HIV) antigen-antibody combination assays: Evaluation of HIV seroconversion sensitivity and subtype detection. J Clin Microbiol 39:3122–3128.

Manns A, Hisada M, La Grenade L. 1999. Human T-lymphotropic virus type I infection. Lancet 353:1951–1958.

Maskill WJ, Crofts N, Waldman E, Healey DS, Howard TS, Silvester C, Gust ID. 1988. An evaluation of competitive and second generation ELISA screening tests for antibody to HIV. J Virol Methods 22:61–73.

Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata K, Hinuma Y. 1981. Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. Nature 294:770–771.

Morgan OS, Rodgers-Johnson P, Mora C, Char G. 1989. HTLV-1 and polymyositis in Jamaica. Lancet 2:1184–1187.

Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M. 1989. Chronic inflammatory arthropathy associated with HTLV-I. Lancet 333:441.

Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M. 1986. HTLV-I associated myelopathy: A new clinical entity. Lancet 1:1031–1032.

Palker TJ, Tanner ME, Scearce RM, Streilein RD, Clark ME, Haynes BF. 1989. Mapping of immunogenic regions of human T cell leukemia virus type I (HTLV-I) gp46 and gp21 envelope glycoproteins with env-encoded synthetic peptides and a monoclonal antibody to gp46. J Immunol 142:971–978.

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77:7415–7419.

Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL. 2005. Global epidemiology of HTLV-I infection and associated diseases. Oncogene 24:6058–6068.

Rodgers-Johnson P, Gajdusek DC, Morgan OS, Zaninovic V, Sarin PS, Graham DS. 1985. HTLV-I and HTLV-III antibodies and tropical spastic paraparesis. Lancet 2:1247–1248.

Shah DO, Chang CD, Jiang LX, Cheng KY, Muerhoff AS, Guttierrez RA, Leary TP, Desai SM, Batac-Herman IV, Salbilla VA, Haller AS, Stewart JL, Dawson GJ. 2003. Combination HCV core antigen and antibody assay on a fully automated chemiluminescence analyzer. Transfusion 43:1067–1074.

Sickinger E, Stieler M, Kaufman B, Kapprell HP, West D, Sandridge A, Deware S, Schochetman G, Hunt JC, Daghtal D, AX SYM Clinical Study Group. 2004. Multicenter evaluation of a new, automated enzyme-linked immunoassay for detection of human immunodeficiency virus-specific antibodies and antigen. J Clin Microbiol 142: 21–29.

Switzer WM, Qari SH, Wolfe ND, Burke DS, Folks TM, Heneine W. 2006. Ancient origin and molecular features of the novel human T-lymphotropic virus type 3 revealed by complete genome analysis. J Virol 80:7427–7438.

Taylor GP, Bodéus M, Courtois F, Pauli G, Del Mistro A, Machuca A, Padua E, Andersson S, Goubau P, Chieco-Bianchi L, Soriano V, Coste J, Ades AE, Weber JN. 2005. The seroepidemiology of human T-lymphotropic viruses: types I and II in Europe: A prospective study of pregnant women. J Acquir Immune Defic Syndr 38:104–109.

Varma M, Rudolph DL, Knuchel M, Switzer WM, Hadlock KG, Velligan M, Chan L, Foung SK, Lal RB. 1995. Enhanced specificity of truncated transmembrane protein for serologic confirmation of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 infections by Western blot (immunoblot) assay containing recombinant envelope glycoproteins. J Clin Microbiol 33:3239–3244.

Vrielink H, Reesink HW, Habibuw MR, Schuller M, van der Meer CBM, Lelie PN. 1999. Comparison of four HTLV-I and HTLV-I + II ELISAs. Vox Sang 76:187–191.

Watanabe T, Mochizuki M, Yamaguchi K. 1997. HTLV-1 Uveitis (HU). Leukemia 11:582–584.

Yakova M, Lézin A, Dantin F, Lagathu G, Olindo S, Jean-Baptiste G, Arfi S, Césaire R. 2005. Increased proviral load in HTLV-1-infected patients with rheumatoid arthritis or connective tissue disease. Retrovirology 2:4–12.

Yamada K, Kuroda N, Washitani Y, Shiraki H, Maeda Y. 1992. Enzyme immunoassay with synthetic peptides to detect anti-HTLV-I antibodies. Clin Chem 38:699–703.

Yamaguchi K, Seiki M, Yoshida M, Nishimura H, Kawano F, Takatsuki K. 1984. The detection of human T cell leukemia virus proviral DNA and its application for classification and diagnosis of T cell malignancy. Blood 63:1235–1240.

Yoshida M, Miyoshi I, Hinuma Y. 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. PNAS 79:2031–2035.