Evaluation of commercial HTLV-1 test kits by a standard HTLV-1 serum panel

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To evaluate the performance of currently available test kits for human T-cell lymphotropic virus type 1 (HTLV-1), we examined two particle agglutination (PA) tests and nine enzyme immunoassays (EIA) using a standard serum panel consisting of HTLV-1-positive and HTLV-1-negative sera that had been characterized by immunofluorescence and the polymerase chain reaction (PCR). The PA kits exhibited 94.0–100.0% sensitivity and 99.5–100.0% specificity; the sensitivity range was ascribed to the quality of the HTLV-1 antigens coated on the particles. The EIA kits had 99.5–100% sensitivity and 98.5–100% specificity; the 98.5%–99.5% specificity exhibited by five of the EIA kits could have been due to non-specific reactions that were detected through use of an inadequate cut-off value and the use of recombinant proteins. It can be concluded that the sensitivity of the currently available PA and EIA kits is sufficient to permit their use for screening purposes; however, the specificity of some EIA kits should be optimized.

Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukaemia (ATL) (*1–3*), HTLV-1-associated myelopathy (HAM/TSP) (*4, 5*) and other HTLV-1-associated diseases (*6–8*). HTLV-1 infections are transmitted via breast-feeding, sexual contact, and blood transfusions (*9*). Exclusion of HTLV-1-contaminated blood has decreased bloodborne transmission of HTLV-1 in Japan (*10*).

Anti-HTLV-1 antibody testing is useful for detecting infection with the virus, because HTLV-1-seronegative persons who are truly infected are rare (*11, 12*). Thus, HTLV-1 test kits for the routine screening of blood donors have become available, but they do not always accurately determine the HTLV-1 status of an individual (*13, 14*).

In order to assess comparatively the various diagnostic assays for HTLV-1 infection that are currently available on the market, we investigated the sensitivity and specificity of particle agglutination (PA) and enzyme immunoassay (EIA) kits for HTLV-1 using the standard HTLV-1 serum panel that we have reported previously (*15*).

The results obtained indicated that most of the kits are sufficiently sensitive to screen for HTLV-1 antibodies, but that some EIA kits need to be optimized to increase their specificity.

Materials and methods

Standard HTLV-1 serum panel

The standard serum panel consisting of sera that were positive or negative for HTLV-1 (standard HTLV-1 serum panel) was established using samples from the HTLV-1 surveillance serum bank in Kagoshima, an HTLV-1-endemic area in southern Japan (*15, 16*). The standard panel consisted of a set of 200 sera from individual asymptomatic HTLV-1 carriers (HTLV-1-positive sera) and 200 sera from healthy donors (HTLV-1-negative sera).

The donors of HTLV-1-positive sera were initially screened using a first-generation PA test (lot 1) (SERODIA ATLA, Fujirebio Inc., Tokyo, Japan) (*17*) and the result confirmed by immunofluorescence test using MT-1 cells (*3*) and by the polymerase chain reaction (PCR) test for HTLV-1 proviral DNAs in their peripheral blood lymphocytes (PBL) using a primer set for the *pX* region (*18, 19*).

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The HTLV-1-negative sera were obtained from normal healthy donors who had no risk of HTLV-1 infection and who were confirmed free of HTLV-1 infection by the serological and virological tests described above.

PA and EIA kits evaluated in the study

Two types of PA kits and nine types of EIA kits were evaluated in the study (Table 1). The PA kits were provided by United Biomedical Inc.[a] (two different lots of the PHA-CHEK test), while Fujirebio[b] provided a second-generation PA kit (lot 2), which differed from the kit that we used previously (*20*). The EIA kits were provided by nine manufacturers.[c] Two different lots of EIA kits were provided by Abbott Laboratories, Diagnostic Biotech Ltd., Organon Teknika Corp., and IAF BioChem International Inc.

The HTLV-1 antigens incorporated into the PA and EIA kits were from different virus strains and cell lines and differed also in their level of purification, as shown in Table 1. All the kits were in satisfactory condition on arrival in Kagoshima and were stored at 4 °C until they were tested.

[a] United Biomedical Inc., New York, NY, USA.

[b] Fujirebio Inc., Tokyo, Japan.

[c] Abbott Laboratories, Abbott Park, Chicago, IL, USA; Genetic Systems Corp., Seattle, WA, USA; Diagnostic Biotech Ltd., Singapore, Singapore; Eisai Corp., Tokyo, Japan; Organon Teknika Corp., Durham, NC, USA; IAF BioChem International Inc., Montreal, Canada; Kyowa Medex Co. Ltd., Tokyo, Japan; Kuraray Co. Ltd., Osaka, Japan; and Cambridge Biotech Corp, Worcester, MA, USA.

The PA and EIA kits were tested according to the manufacturers' instructions and the sensitivity and specificity were evaluated using the HTLV-1-positive and HTLV-1-negative sera. The δ-value (mean $\log_{10}$ [sample reading/cut-off value]/standard deviation) developed by Crofts et al. (*21*) was calculated for each EIA kit.

Results

The standard HTLV-1 serum panel

For the study we used the standard HTLV-1 serum panel that had been established using serological and virological methods at the WHO Collaborating Centre for Human Retroviral Infections associated with Neurological Disorders, Kagoshima University. We selected 200 HTLV-1 positive sera from 1309 asymptomatic HTLV-1 carriers who had been followed up by the HTLV-1 surveillance programme in Kagoshima prefecture since 1985 (*15*). For the HTLV-1-positive sera the anti-HTLV-1 antibody titres lay in the range 32–65 536 by PA test. The HTLV-1-negative sera were obtained from 200 healthy blood donors in Kagoshima prefecture who were negative for anti-HTLV-1 antibodies and whose PBL were negative for proviral DNA (Table 2).

Sensitivity and specificity of the PA kits

Two different lots of the PHA-CHEK kit and one lot of the SERODIA HTLV-I kit (lot 2) were examined for their sensitivity and specificity using the standard HTLV-1 serum panel. Lot 1 and lot 2 of the PHA-

Table 1: **Characteristics of the particle agglutination (PA) and enzyme immunoassay (EIA) kits for HTLV-1 antibodies evaluated in the study**

	Manufacturer	Type of antigen
PA kits		
PHA-CHEK (lots 1 and 2)	United Biomedical Inc., USA	HTLV-1 lysate/synthetic peptide (env)
SERODIA HTLV-I[a] (lot 2)	Fujirebio Inc., Japan	HTLV-1 lysate (TCL-Kan)/enriched protein (gp46)
EIA kits		
ABBOTT HTLV-I EIA (lots 1 and 2)	Abbott Laboratories, USA	HTLV-1 purified virion (HUT 102.B2. cell)
GENETIC SYSTEMS HTLV-I EIA	Genetic Systems Corp., USA	HTLV-1 purified virion (HB3 cell line)
HTLV-I ELISA KIT (lots 1 and 2)	Diagnostic Biotech. Ltd., Singapore	HTLV-1 purified virion (MT-2 cell line)
NEW EITEST-ATL	Eisai Corp., Japan	HTLV-1 purified virion (MT-2 cell line)
VIRONOSTIKA HTLV-I (lots 1 and 2)	Organon Teknika Corp., USA	HTLV-1 viral lysate (MT-2 cell line)
DETECT-HTLV (lots 1 and 2)	IAF BioChem, International Inc., Canada	Synthetic peptide (gp46)
DETERMINER HTLV-I ANTIBODY	Kyowa Medex Co. Ltd., Japan	Recombinant protein (p24 and gp46)
K ASSAY ANTI HTLV-I	Kuraray Co. Ltd., Japan	Synthetic peptides (p19 and gp46)
RECOMBINANT HTLV-I/II EIA	Cambridge Biotech Corp., USA	Recombinant protein (gp21)

[a] Different lot from that used to establish the standard HTLV-1 panel sera.

Table 2: **Characteristics of the standard HTLV-1 serum panel**

Panel	Screening test titre[a]	No. of sera	Supplemental test: IF[b]	Supplemental test: PCR[c]
HTLV-1 positive sera[d] (n = 200)	× 32	2	+ve	+ve
	× 64	10	+ve	+ve
	× 128	26	+ve	+ve
	× 256	45	+ve	+ve
	× 512	31	+ve	+ve
	× 1024	28	+ve	+ve
	× 2048	28	+ve	+ve
	× 4096	17	+ve	+ve
	× 8192	8	+ve	+ve
	× 16 384	1	+ve	+ve
	× 32 768	3	+ve	+ve
	× 65 536	1	+ve	+ve
HTLV-1 negative sera[e] (n = 200)	<×16	200	–ve	–ve

[a] Particle agglutination test. HTLV-1 antibody titres. PA positivity was defined by agglutination at ≥16-fold serum dilution.
[b] Immunofluorescence. Positivity was defined by positive immuno-staining at a 10-fold serum dilution.
[c] Polymerase chain reaction. Positivity was defined by nested PCR of the *pX* region.
[d] Obtained from 200 asymptomatic carriers.
[e] Obtained from 200 normal healthy donors.

CHEK kit had low sensitivity, 95.0% and 94.0%, respectively; and the SERODIA HTLV-I kit (lot 2) had 100% sensitivity to the HTLV-1-positive sera (Table 3, PA kits).

Lot 2 of the PHA-CHEK kit reacted with one of the 200 HTLV-1-negative sera, corresponding to a specificity of 99.5%. Lot 1 of the PHA-CHEK kit and lot 2 of the SERODIA HTLV-I kit had a specificity of 100% to the HTLV-1-negative sera (Table 3, PA kits).

Sensitivity and specificity of the EIA kits

The sensitivity of the EIA kits was 100% except for the ABBOTT HTLV-I EIA (lot 2) and DETERMINER HTLV-I ANTIBODY tests, both of which had 99.5% sensitivity (Table 3, EIA kits).

The specificity of the EIA kits ranged from 98.5% to 100% (Table 3, EIA kits). Five kits (GENETIC SYSTEMS HTLV-I EIA, DETECT-HTLV, DETERMINER HTLV-I ANTIBODY, K ASSAY ANTI HTLV-I and RECOMBINANT HTLV-I/II EIA) reacted with some HTLV-1-negative sera (Table 4). The nonspecific reactions were reproduced by triplicated experiments. The DETERMINER HTLV-I ANTIBODY kit displayed a relatively high nonspecific reaction with one serum (N-173).

Evaluation of EIA kits by δ-value

Based on the positive δ-values, the GENETIC SYSTEMS HTLV-I EIA had the highest (δ= 17.919) and the ABBOTT HTLV-I EIA (lot 2) the lowest (δ = 4.731) (Table 5, positive column). The negative δ-values indicated that ABBOTT HTLV-I EIA (lot 2) had the highest specificity (δ = –6.203) and DETECT-HTLV (lot 2) the lowest (δ = –2.300) (Table 5, negative column).

Discussion

Usefulness of the standard HTLV-1 serum panel

Geographical subtypes of HTLV-1 have been identified in molecular studies of HTLV-1 sequences. For

Table 3: **Sensitivities and specificities of the particle agglutination (PA) and enzyme immunoassay (EIA) kits**

	Sensitivity (%) (n = 200)	Specificity (%) (n = 200)
PA kits		
PHA-CHEK		
Lot 1	95.0	100.0
Lot 2	94.0	99.5
SERODIA HTLV-I	100.0	100.0
Lot 2		
EIA kits		
ABBOTT HTLV-I EIA		
Lot 1	100.0	100.0
Lot 2	99.5	100.0
GENETIC SYSTEMS	100.0	98.5
HTLV-I EIA		
HTLV-I ELISA KIT		
Lot 1	100.0	100.0
Lot 2	100.0	100.0
NEW EITEST-ATL	100.0	100.0
VIRONOSTIKA HTLV-I		
Lot 1	100.0	100.0
Lot 2	100.0	100.0
DETECT-HTLV		
Lot 1	100.0	100.0
Lot 2	100.0	99.5
DETERMINER HTLV-I	99.5	99.5
ANTIBODY		
K ASSAY ANTI HTLV-I	100.0	99.5
RECOMBINANT	100.0	99.3
HTLV-I/II EIA[a]		

[a] For this kit, the sensitivity and specificity were evaluated using 147 and 136 samples, respectively.

Table 4: **Details of the nonspecific reactivity exhibited by five of the HTLV-1 EIA kits**

	Serum sample	Test	OD[a]	Cut-off	OD ratio[b]	Assessment	WB[c]	PCR[d]
GENETIC SYSTEMS	N-012	1	0.552	0.402	1.37	+	p24+	–
HTLV-I EIA		2	0.535	0.405	1.32	+		
	N-175	1	0.592	0.416	1.42	+	p19+	–
		2	0.492	0.409	1.20	+		
		3	0.478	0.402	1.19	+		
	N-195	1	0.498	0.402	1.24	+	No	–
		2	0.392	0.409	0.96	–	band	
		3	0.457	0.409	1.12	+		
DETECT-HTLV	N-144	1	0.128	0.124	1.03	+	No	–
Lot 2		2	0.082	0.125	0.66	–	band	
		3	0.134	0.124	1.08	+		
RECOMBINANT	N-061	1	0.709	0.380	1.87	+	p24+	–
HTLV-I/II EIA		2	0.499	0.337	1.48	+		
K ASSAY	N-192	1	0.263	0.137	1.92	+	No	–
ANTI HTLV-I		2	0.495	0.148	3.34	+	band	
		3	0.323	0.148	2.18	+		
DETERMINER HTLV-I	N-173	1	0.788	0.150	5.25	+	No	–
ANTIBODY		2	0.800	1.164	4.88	+	band	
		3	0.793	0.174	4.56	+		

[a] Optical density.
[b] OD/cut-off.
[c] Western blot.
[d] Polymerase chain reaction.

Table 5: **δ-Values for the EIA kits according to their reactions with HTLV-1-positive and HTLV-1-negative sera**

	δ-value of panel:	
	Positive	Negative
ABBOTT HTLV-I EIA		
Lot 1	8.264	–5.116
Lot 2	4.731	–6.203
GENETIC SYSTEMS HTLV-I EIA	17.919	–2.767
HTLV-I ELISA KIT		
Lot 1	6.283	–2.535
Lot 2	7.671	–3.506
NEW EITEST-ATL	10.485	–3.547
VIRONOSTIKA HTLV-I		
Lot 1	10.677	–4.238
Lot 2	9.577	–4.745
DETECT-HTLV		
Lot 1	8.833	–3.423
Lot 2	6.119	–2.300
DETERMINER HTLV-I ANTIBODY	5.305	–2.527
K ASSAY ANTI HTLV-I	6.536	–3.335
RECOMBINANT HTLV-I/II EIA	4.981	–2.992

example, Ehrlich et al. categorized four major subtypes of HTLV-1 isolates: HTLV-1 (PNG-1) (Papua New Guinea); HTLV-1 (EL) (Africa); and two Japanese HTLV-1 variants — HTLV-1 (ATK, 4C) and HTLV-1 (MT-2) (*22*). The Japanese variants prevail in Kagoshima prefecture (*23, 24*). The standard HTLV-1 serum panel used in the study was obtained from the epidemiologically proven HTLV-1-endemic area; however, there was no significant variation in the immunoreactivity with the standard HTLV-1 serum panel obtained from donors in Kagoshima as reported in this study. This result is consistent with evidence that immune responsiveness to the HTLV-1 *env* region is conserved among diverse geographical populations, as reported by Buckner et al. (*25*) and Lal et al. (*26*). Conservation of the immunodominant epitopes of the HTLV-1 *env* region has also been confirmed by sequence analysis of the four major subtypes of HTLV-1 (*27, 28*).

Sensitivity and specificity of the PA kits

The low sensitivity of the PHA-CHEK kit seemed to be caused by the limited number of antigenic epitopes when synthetic peptides of the HTLV-1 *env* region are used. Different immunodominant epitopes of the HTLV-1 *env* region have been described (*29–31*). Thus, the PHA-CHEK kit, which uses only

one *env* epitope, might have a low sensitivity for detecting anti-HTLV-1 antibodies. In fact, the highest sensitivity was yielded by the SERODIA HTLV-I kit (lot 2), which incorporates HTLV-1 whole virion antigens and enriched whole *env* proteins (Table 3).

Sensitivity and specificity of the EIA kits

All the EIA kits had high sensitivity to the HTLV-1-positive sera, except the ABBOTT HTLV-I EIA (lot 2) and DETERMINER HTLV-I ANTIBODY tests (Table 3). The ABBOTT HTLV-I EIA lot 2 failed to detect one of the positive sera that had a 128-fold titre of anti-HTLV-1 antibody by PA test (SERODIA, lot 1). This failure appears to have been caused by poor quality control during the manufacture of the kits, because the serum in question exhibited a positive reaction with lot 1 of the ABBOTT HTLV-I EIA. The DETERMINER HTLV-I ANTIBODY test failed to detect another positive serum, which had a 256-fold titre of anti-HTLV-1 antibody in the PA test (SERODIA, lot 1) and exhibited relatively low immunoreactions with p24 and gp46 in Western blots. This could have been caused by the smaller number of the HTLV-1 antigens and the consequent lower reactivity.

The nonspecific reactions of seven of the HTLV-1-negative sera in five of the EIA kits could have arisen for two reasons. First, the cut-off values with the GENETIC SYSTEMS HTLV-I EIA and DETECT HTLV-I tests were insufficient to exclude nonspecific reactions (Table 4). Second, they could have been due to the presence of contaminants in the HTLV-1 recombinant proteins used in the DETERMINER HTLV-I ANTIBODY and RECOMBINANT HTLV-I/II EIA kits. Both these kits used *Escherichia coli* to develop the HTLV-1 recombinant proteins. The seven sera in question did not produce any nonspecific reactions with any of the remaining eight EIA kits, which exhibited 100% specificity (Table 3).

All the EIA kits were evaluated using the δ-value, which is based on the reactivity with the standard HTLV-1 serum panel and the cut-off value used (*21*). There were no significant differences between the positive δ-values. The negative δ-values were relatively low for the five EIA kits with 98.5–99.5% specificity.

In conclusion, the sensitivity of the currently available EIA kits is sufficient to screen for anti-HTLV-1 antibodies and there was no significant difference among the kits that used HTLV-1 antigens, purified virion, recombinant proteins or synthetic peptides; however, some kits need to be optimized to increase their specificity.

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Résumé

Evaluation des trousses commerciales HTLV-1 au moyen d'une batterie normalisée de sérums anti-HTLV-1

Le HTLV-1 (*human T-cell lymphotropic virus type 1*) est l'agent étiologique de la leucémie à cellules T de l'adulte (ATL), de la myélopathie associée au HTLV-1 (HAM/TSP) et d'autres maladies associées à ce virus. Le HTLV-1 se transmet par le lait maternel, par contact sexuel et par transfusion sanguine. L'exclusion des dons de sang contaminés par le HTLV-1 a réduit la transmission de ce virus par voie sanguine au Japon.

La sérologie HTLV-1 est utile pour dépister les infections à HTLV-1, car les sujets infectés sont rarement séronégatifs à l'égard de ce virus. Les trousses HTLV-1 peuvent donc être utilisées pour le dépistage systématique des donneurs de sang, mais ne permettent pas toujours de déterminer avec exactitude le statut du sujet vis-à-vis de l'infection par le HTLV-1.

Nous avons comparé au moyen d'une batterie normalisée de sérums anti-HTLV-1 les diverses trousses de diagnostic de l'infection à HTLV-1 disponibles sur le marché, qui se divisent en gros en deux types: les tests basés sur l'agglutination de particules (PA) et les titrages immuno-enzymatiques (EIA). Pour effectuer cette comparaison, nous avons utilisé 200 sérums positifs pour le HTLV-1 et 200 sérums négatifs pour le HTLV-1, prélevés chez des habitants de la préfecture de Kagoshima (Japon), une région connue d'endémie à HTLV-1.

Nous avons évalué 2 trousses PA et 9 trousses EIA. Les trousses PA avaient une sensibilité de 94,0–100,0% et une spécificité de 99,5–100,0%. Les différences de sensibilité observées avec les tests PA ont été attribuées à la qualité de l'antigène HTLV-1 utilisé. Les trousses EIA avaient une sensibilité de 99,5–100,0% et une spécificité de 98,5–100,0%. La spécificité de

98,5–99,5% de certains tests EIA peut s'expliquer par la comptabilisation de réactions non spécifiques due au choix d'un seuil inadapté et par l'emploi de protéines recombinantes. Toutes les trousses EIA ont également été évaluées au moyen d'un autre paramètre statistique, la valeur δ.

Nos résultats indiquent que la sensibilité des trousses PA et EIA actuellement disponibles sur le marché est suffisante aux fins de dépistage, mais que la spécificité de certaines trousses EIA doit être optimisée.

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