FEASIBILITY OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I SCREENING USING POOLED SERA

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Background and Purpose: The human T-cell lymphotropic virus type I (HTLV-I) seroprevalence rate among volunteer blood donors in Taiwan is low. To study the feasibility of HTLV-I enzyme immunoassay (EIA) screening using pooled sera, we prospectively compared its sensitivity to that of the routine test for each donor.

Methods: HTLV-I EIA tests for each serum sample and a pooled-sera test with 50 samples from voluntary donated blood samples were performed concurrently to assess the effectiveness and cost savings of this screening method.

Results: Of 135,606 blood samples from volunteer donors tested for HTLV-I infection, 60 samples (0.044%) were HTLV-I EIA-positive using the routine method. Among these, the positive results were confirmed by Western blot in 22 samples (36.7%). In the pooled-sera test, 17 of 2,713 pooled samples were EIA-positive and these results were all confirmed by Western blot. Five of the 22 (22.7%) EIA-positive samples had a false-negative result in the pooled-sera test. Serial dilution in these five cases revealed that the maximum dilution before loss of sensitivity was 8-fold for two specimens and 16-fold for three specimens.

Conclusion: In this study, the 50-pooled sera test had higher specificity (100%), but lower sensitivity (77.3%), than the routine HTLV-I screening. Our results suggest that use of a pooling method with five samples would leave a reasonable safety margin and be feasible for HTLV-I mass screening in areas with low seroprevalence for HTLV-I infection.

Infection with human T-cell lymphotropic virus types I and II (HTLV-I/II) is commonly associated with tropical spastic paraparesis [1], adult T-cell leukemia/lymphoma, and some inflammatory disorders [2, 3]. Although the virus is primarily sexually transmitted, it may also be transmitted from mother to child perinatally, during breast-feeding, or through blood transfusion [4–6]. To prevent HTLV-I/II-associated disorders, screening of each volunteer blood donor sample becomes important in routine blood banking. Serologic tests for HTLV-I/II became available in 1986, and HTLV-I/II screening in volunteer blood donors became routine in Japan in 1986, in the USA in 1988, and in France in 1991 [7, 8]. Epidemiologic studies have found HTLV-I prevalences of 0.3 to 40% among different populations [9–13].

Routine screening for HTLV-I/II infection in volunteer blood donors became mandatory in every blood donation center in Taiwan beginning in February 1996. Our previous study found a low HTLV-I seroprevalence in volunteer blood donors in Taiwan [14]. According to the annual report of the Chinese Blood Services Foundation in Taiwan, about 1.92 million blood units were collected from voluntary donors in 2000. The cost-effectiveness of HTLV-I screening for every volunteer donor in an area of such low prevalence has been questioned. Our observations of HTLV-I enzyme immunoassay (EIA) results found a high optical density (OD, > 3.0) in HTLV-I immunoassay in Western blot (WB)-confirmed samples.

This study assessed the practicality of using pooled sera in routine screening for HTLV infection. Dilution testing was performed initially to find the appropriate number of serum samples for pooling, followed by a prospective study to compare results using the routine test and pooled sera.
Materials and Methods

All voluntary blood donors at the Kaohsiung Blood Center from December 1997 to May 1998 were included in the study. Each donor sample underwent EIA to test for HTLV-I/II, HIV (Murex Wellcozyme HIV 1+2 v 54/55; Murex Biotech Ltd., Dartford, U.K.), hepatitis B surface antigen (Murex HBsAg GE14/15/16; Murex, Dartford), and antibodies to hepatitis C virus (anti-HCV; SP-NANBASE C-96; Murex, Kyalami, Republic of South Africa), further testing for alanine aminotransferase (ALT; IFCC, Olympus Diagnostica GmbH, Lismeehan, Ireland), and the reversed passive hemagglutination test (Biokit; Fujirebio, Taoyuan, Taiwan). HTLV-I EIA screening was performed using the Murex HTLV I+II, GE80/81 kit (Murex, Dartford) according to recommended procedures. The status of reproducibly positive samples was confirmed by WB performed using the HTLV Blot 2.4 assay kit (Genelabs Diagnostics (Pte) Ltd., Singapore). Test sera were considered HTLV-I seropositive when reactivity was found to Gag (p19 with or without p24) and Env (GD21 and rgp46-I), and HTLV-II seropositive when reactivity was found to Gag (p24 with or without p19) and Env (GD21 and rgp46-II).

Serum dilution

A pilot test to determine the largest number of samples that could be pooled without loss of sensitivity assessed the dilution at which 19 WB-confirmed samples taken from before December 1997 had an EIA OD greater than 3.0 with a cutoff value of positivity around 0.25 OD. Sensitivity was lost at dilutions between 1:64 and 1:2,048. Hence, 50 samples was set as the maximum for the prospective study to compare pooled-sera screening with routine EIA screening. A binary screening method was used to find positive samples within 50-pooled positive sera samples (Figure). The predicted value was compared with results obtained using the routine method.

Pooled sera test

Two technicians were assigned to perform the tests: one performed the routine test for each sample and the other performed the 50-pooled sera test and dilution test for EIA-positive pooled samples. The results obtained by both methods were compared. All WB-positive samples were also subjected to sera dilution test to find the maximal dilution power.

Results

A total of 135,606 volunteer blood samples collected from December 1997 to May 1998 were included in the study. Among these, 60 samples (0.044%) were positive on routine EIA screening, 22 (36.7%) of which were confirmed by WB. In the pooled-sera method, 17 of 2,713 pooled samples were positive for HTLV-I. These findings were compatible with those of routine screening. Of the 22 samples confirmed by WB on routine testing, five (22.7%) were falsely EIA-negative in the pooled-sera test (Table 1). The sensitivity and specificity of the 50-pooled sera test were 77.3% and 100%, respectively. The results of serial dilution tests in the 41 WB-positive samples (including the 19 positive samples collected before December 1997) revealed that HTLV-I could be detected by EIA at dilutions ranging from undiluted to 2,048-fold (Table 2). All five falsely negative samples by pooled-sera test had low EIA dilution titers (2 eight-fold and 3 16-fold). The confirming p19/ p24, GD21, and rgp46-I bands in these samples were significantly weaker than those in positive cases.

Discussion

Our previous study showed a low seroprevalence (0.06%) of HTLV-I infection, as assessed by WB, in volunteer blood donors in Taiwan [14]. Due to the need for cost-effective mass screening, methods using
Pooled sera have been advocated. Pooling methods have been assessed in the blood banking system, especially in testing for the HIV antibody, which is as sensitive and specific as individual sample testing [15–18]. There have been few reports of HTLV-I screening using pooled sera [19]. The main question in selecting a pooling method is how many samples can be pooled without a loss of sensitivity. Although a maximum pool of four to five samples is recommended in areas where the seroprevalence of HIV is less than 2% [15, 18], there is a lack of data regarding the use of pooled samples in HTLV-I screening. Andersson et al evaluated a pooling strategy for HTLV-I/II screening using four antibody screening assays. Their data suggest that five samples would provide a reasonable and safe pooling method [19].

The present study found that the sensitivity and specificity of a 50-pooled test were 77.3% and 100%, respectively. Among the five false-negative samples, all had dilution powers of less than 1:64. These results indicate that less than eight samples should be pooled. Thus, our results confirm previous findings that five samples would provide a reasonable and safe pooling method for HTLV-I screening.

Due to the low risk of disease transmission via HTLV-I-infected donor blood [7, 20–22], it is worth reassessing the feasibility of routine screening in the blood banking system in areas of low HTLV-I seroprevalence. The most cost-effective HTLV-I screening method for routine use in blood banking remains to be determined. Although several screening strategies, including screening only in new blood donors or detection with polymerase chain reaction, could be used for HTLV-I screening [8, 23], our results indicate that a pooling method with five samples is feasible for HTLV-I mass screening in areas with low seroprevalence of HTLV-I infection, at a cost of only about one-fifth of the routine cost of screening.

In conclusion, we found that a 50-pooled-sera test had a higher specificity, but lower sensitivity, than the routine HTLV-I screening test used in blood banking. Use of a pooling method with five samples would leave a reasonable safety margin and be feasible for mass screening in areas with low seroprevalence for HTLV-I infection.

### References

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