there was peripheral agglutination of the particles in a clear large ring with a rough multiform outer margin (+), and 10 in which a film of agglutinated particles was spread out uniformly on the bottom of the well (++). Table 1 also shows the donor locations of these 29 breast milk samples: seven from Okinawa, three from Nagasaki, three from Hyogo, three from Miyagi, and eight from Hokkaido were positive. Samples from all other Japanese regions were negative. Five samples (1.8%) from the other countries required confirmation, with two from Beijing, one from Hanoi, and two from Seoul being positive.

HTLV-1 and HTLV-2 were examined using the Innogenetics™ Inno-lia™ HTLV I/II Score using 10-fold quantities of samples according to the manufacturer's standard protocol. As shown in Table 1, two samples were positive and four had a deferred pattern. As shown in Fig. 1, a positive pattern for HTLV-2 was observed in one sample from China, and the sample did not react to gp46-175, suggesting that the donor may be an HTLV-2 carrier. Although it is difficult to distinguish between HTLV-1 and HTLV-2 using the PA method, Berini *et al.*[12] reported that the PA sensitivity of HTLV-2 in the blood of carriers is equal to that of HTLV-1.

The incidence of HTLV-1 infection is high in western Japan, particularly in Kyushu,[3–5,13] with some cases also found in Hokkaido,[14] and it has also been reported in equatorial Africa, the Caribbean islands, Colombia, Brazil, south India, Papua New Guinea, northeast Australia, and among indigenous people living at the margins of the Andes plateau in South America, which represent endemic areas of the virus.[2] As shown Table 1, with the exception of Japan, HTLV-1-endemic areas are often in developing countries, where blood testing may not be feasible. The PA method uses a freeze-dried product that is prepared when required, and determination is made by diluting the sample and visually observing agglutination images; thus, it can easily be used for preliminary screening and is effective for use in developing countries.

It seems that the PA positivity ratio in breast milk is higher than that in the blood, but the results of the LIA method in the present study were equivalent to those obtained with blood samples. Thus, although the PA method using breast milk is useful as a first screen, follow-up testing is necessary.

The sp-ELISA method uses ELISA-coated synthetic peptides corresponding to the immunodominant regions of HTLV-1 structural proteins,[11] and for this study we used p19 gag protein (100 to 130 aa), gp46 protein (175 to 199 aa), and gp46 (288 to 317 aa). The peptide corresponding to the region of the Env gp46 protein, 175 to 199 aa (gp46-175), allows the discrimination of HTLV-1/2 antibodies.

HTLV-2 has been isolated from individuals other than leukemia patients, and its association with disease remains unclear. It has also been found in locations including Central and South America,[7] Sweden,[15] Spain,[16] and Brazil,[17] and its roots are believed to lie in the indigenous peoples of areas such as the Florida peninsula of the United States, the Yucatan peninsula of Mexico, and Panama.[5] In this study, it was interesting that we detected an HTLV-2-positive infectant in China.

In countries such as Japan, the United States, France, and the Netherlands, blood donations are routinely checked for a variety of infectious diseases. Recently, China[18] and Korea[19] have reported epidemiological analyses of HTLV-1 and -2 infection, but in other Asian countries, screening has only been established in the last few years, if at all.[20] High-performance antibody screening, such as that on donated blood, is required to assess the status of HTLV infection and to prevent further infection. While it is useful to demonstrate the validity of screening breast milk as opposed to blood, it is important not only to facilitate simpler screening but also highly accurate screening. Further studies on the sensitivity, specificity, and reliability of assays using antibodies to HTLV-1 and -2 in breast milk are necessary for large-scale epidemiological surveys of HTLV infection.

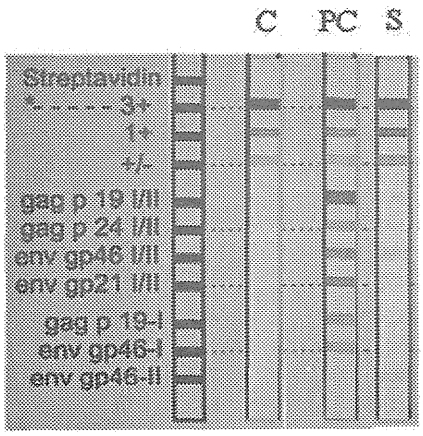

Fig. 1. Positive LIA Reactions of Milk Samples

C, control; PC, positive control; S, milk sample (HTLV-2 (±)).

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