RESULTS OF ELISA SCREENING FOR ANTIBODIES TO HTLV-III IN 1014 HEALTHY BLOOD DONORS FROM NORTHERN CALIFORNIA

ELISA P/N ratio*					
<2	2–3·9	4–5·9	6–7·9	8–9·9	>10
921	75†	12	4	2	0

*Median P/N ratio = 0·7, mean±SD = 0·95±0·99
†Single true positive serum (P/N ratio 2·7).

development of a screening test to detect antibodies to AIDS-related retroviruses. The US Public Health Service has recommended excluding blood donors known to be at high risk of AIDS. With the introduction of an enzyme-linked immunosorbent assay (ELISA) for antibody to human T-lymphotropic virus type III (HTLV-III) regulations will soon require the screening of all blood donors. As with any screening test, the problem lies with false positives which will have a significant impact both on blood supplies and on blood donors since seropositive blood will be discarded and donors will be notified of their test result. The definition of "positivity" is thus an important issue. The positive detection limit is best established by comparison of the ELISA P/N ratio with reference methods: the ELISA P/N ratio is calculated as the optical density of a test specimen divided by that of the background or a negative sample. To establish performance standards we compared results by ELISA with those obtained by immunofluorescent assay (IFA) and western blot procedure. The target antigen was gradient purified, disrupted HTLV-III for ELISA and western blot and productively infected cells for IFA. The HTLV-III infected cell line was provided by Dr R. C. Gallo.

We screened 1014 consecutive anonymous blood donor sera by ELISA and retested all specimens with P/N ratios of 2 or more by IFA and western blot (table). Our regional blood centre serves a population of 1·5 million and draws 77 000 units a year from about 50 000 individuals in twelve counties of northern California, excluding San Francisco County. A large percentage of the blood is drawn in Sacramento County where 13 cases of AIDS have been reported since 1982. 2 additional cases have been reported in the other eleven counties. The general donor population thus appears to be at low risk of AIDS.

93 specimens (9·2%) had P/N ratios of 2 or more by ELISA. These were re-examined by IFA and western blot and 1 serum was found (P/N ratio 2·7) which contained antibodies to HTLV-III. Virus specificity was confirmed in the western blot by reactivity with HTLV-III polypeptides (p61, p54, p41, p24).[2] The remaining 92 sera were negative by IFA and western blot. This included 18 specimens with an ELISA P/N ratio of 4·0 or more. None of 48 selected samples with P/N ratios below 2 contained HTLV-III antibodies as identified by IFA or western blot.

Blood banks want to be able to identify all true-positive results without jeopardising the blood supply by unnecessarily deferring blood donors or alarming donors by mentioning a "positive" test that does not represent true infection. In a recent study of a blood donor population, a P/N ratio of 5·0 was established as the cut-off for true positives.[3] However, none of the specimens with a P/N ratio ⩽4·0 were examined by confirmatory methods. Therefore, according to our findings true positives may have been missed in that study. Our results indicate that use of the more sensitive P/N ratio of 2 as a cut-off point without confirmatory testing would have resulted in 9·2% of blood units being discarded. However, only a single unit would have been discarded if ELISA screening had been used in combination with a confirmatory test.

We conclude that it is necessary to use the most sensitive ELISA P/N value possible to detect all antibody-positive sera in the healthy blood donor population. When used in combination with a confirmatory test, either IFA or western blot, this strategy will not result in a major disruption in the procurement of blood or in the significant loss of future blood donors. Further, we recommend that only individuals who are positive by both ELISA and a confirmatory test be placed on a deferred donor list and informed about their AIDS serology results.

A few symptomless virus-positive individuals without antibody will be missed by even the most sensitive HTLV-III antibody screening methods.[4] The resolution of this problem depends on HTLV-III antigen detection tests yet to be developed.

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1 CDC. Provisional Public Health Service interagency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. *MMWR* 1985; **34**: 1–5
2 Sarngadharan MG, Popovic M, Bruch L, et al. Antibodies reactive with human T-cell leukemia retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* 1984; **224**: 506–08
3 Weiss SH, Goedert JJ, Sarngadharan MG, et al. Screening test for HTLV-III (AIDS agent) antibodies. *JAMA* 1985; **253**: 221–25
4 Salahuddin SZ, Groopman JE, Markham PD, et al. HTLV-III in symptom-free seronegative persons. *Lancet* 1984; i: 1418–20.

SIR,—We believe that current commercial kits for HTLV-III antibody tests are likely to give a high rate of false-positive results. We would therefore recommend that careful consideration be given before they are introduced for the screening of all voluntary blood donors, for the amount and degree of unnecessary stress and hardship that a fair number of our donors and their families would thus have to undergo is unacceptable. This in turn could lead to a sizeable drop in the supply of blood and blood products. Of no less importance, for the safety of transfused patients, is the need to ensure that the first priority for the introduction of any HTLV-III antibody tests into a community is given to patients attending special (venereal disease) clinics and other members of the general public who wish to have access to these tests. If this is not done, many high-risk people, from a blood-transfusion point of view, may present themselves at blood-donation sessions simply to find out their HTLV-III antibody status.

We do support, strongly, the screening of all blood donors for HTLV-III antibody testing, but we would advise that this is delayed until test systems have been appropriately evaluated and efforts have been made to give all members of the public access to HTLV-III antibody testing.

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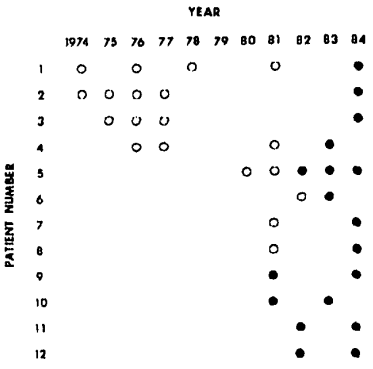
HTLV-III ANTIBODY IN SEQUENTIAL PLASMA SAMPLES: FROM HAEMOPHILIACS 1974–84

SIR,—In an earlier report[1] we showed that seropositivity for antibody to human T-lymphotropic virus type III (HTLV-III) among Scottish and Danish haemophiliacs was related to their use of factor concentrate products made from United States donor material. We here present the HTLV-III antibody results on



HTLV-III antibody status on serial plasma samples from Scottish haemophiliacs.

Ratios above 5·0 were considered positive for the enzyme-linked immunoassay method used.[2]

sequentially collected plasma (1974–84) from the twelve Scottish patients who were positive in our earlier study in 1983–84. All patients were long-term users of factor concentrate (mean age 26 years, range 15–42).

As the figure shows, patients 9–12 were seropositive on both occasions for which plasma was available, and none were tested before 1981. However, seroconversion was recorded in the remaining eight patients and took place after 1981 in at least six of them (there were no samples for the period 1978–83 in patients 2 and 3, so we know only that these patients became HTLV-III antibody positive after 1977).

These data and others from American haemophiliacs (J. J. Goedert, personal communication) support the hypothesis that infection with HTLV-III is new in the haemophilic environment. More specifically, our study suggests that the virus was introduced into Scotland at about the same time as the onset of the AIDS epidemic.

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WILD AFRICAN GREEN MONKEYS OF BARBADOS ARE HTLV NEGATIVE

SIR,—It has been suggested[1,2] that human T-cell leukaemia virus (HTLV) originated in Africa and was distributed to what are now adult T-cell leukaemia/lymphoma (ATLL) endemic areas of south-west Japan and the Caribbean by Portuguese sailing ships crewed by African slaves[3] or by interspecies transmission from Old World primates.[1,2]

The first recorded contingent of Black Africans arrived on Barbados in 1627, and by 1830 an estimated 353 000 Africans had been transported to the island.[6] Coincident with the slave trade, African green monkeys were brought to Barbados, and by 1680 were already pests destroying crops. Today, Barbados has a wildlife management and trapping programme to reduce crop losses, and captured monkeys are bled on the day of capture at the Primate Centre and their sera are stored frozen. HTLV antibodies have been detected in captive African green monkeys (*Cercopithecus sabaeus*) in Germany[4] and other macaque species of Asia and Africa.[2,4,5]

125 serum samples from 74 adult (34 male) and 51 juvenile (below 4 years, 27 male) monkeys captured from different areas of Barbados and selected to give an island-wide profile were heat inactivated at 56°C for 30 min and screened for HTLV antibodies at a 1:10 dilution in phosphate-buffered saline two or three times on different lots of methanol-fixed MT-2 cells,[7] with positive and negative controls, by indirect immunofluorescence. No serum was HTLV antibody positive.

Naturally occurring HTLV antibodies have been detected in free-ranging Japanese macaques (*Macaca fuscata*),[8] suggesting a possible reservoir for human HTLV infection. However, further studies detected seronegative troops of Japanese monkeys in ATLL endemic areas and seropositive troops in non-endemic areas. Our data provide further epidemiological evidence that Old World non-human primates infected with HTLV or HTLV-like virus are not likely to be reservoirs for human HTLV infection.

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ASPARTAME MAY IMPERIL DIETARY CONTROL OF PHENYLKETONURIA

SIR,—A child with well-controlled phenylketonuria (PKU) showed unexpectedly high serum phenylalanine levels. At a birthday party he had drunk five 25 cl bottles of soft drinks sweetened with aspartame. This artificial sweetener, a methyl ester of aspartyl-phenylalanine, is hydrolysed by intestinal esterases and dipeptidases to methanol, aspartate, and phenylalanine. The aspartate is transformed in large part to carbon dioxide via the tricarboxylic acid cycle and the phenylalanine is normally converted to tyrosine or incorporated into body protein.[1] However, homozygous patients with classical PKU have a defect in the phenylalanine hydroxylase, the enzyme that is essential for converting phenylalanine to tyrosine. As a result phenylalanine levels increase after birth and mental retardation ensues if the condition is not treated.[2] The aim is a phenylalanine-restricted diet compatible with normal growth and development but low enough to ensure that serum phenylalanine levels do not rise above 3–10 times the normal level (ie, the range 3–10 mg/dl for children of school age). Generally, children with classical PKU tolerate 10–15 mg phenylalanine per kg body weight per day at 5 years of age.[3]