Comparison of assays for antibody to HTLV I

PHILIPPA M B WHITE *Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London*

SUMMARY Indirect immunofluorescence, competitive radioimmunoassay, HTLV I-enzyme linked immunosorbent assay and gelatin particle agglutination Serodia-ATLA were compared in terms of their ability to detect antibody to human T cell leukaemia virus I (HTLV I). The sensitivities were 96.9%, 92%, 97.0%, and 100%, respectively, and the specificities 99.3%, 98.9%, 98.6%, and 96.3%. Particle agglutination was very simple to perform and was the most sensitive, though the least specific test. Antibody titres were 10-100 times higher when measured by particle agglutination than by other tests, and antibody titres were considerably higher in patients with neurological disease related to HTLV I than in those with other conditions.

Serodia-ATLA is the method of choice for preliminary screening of specimens for antibody to HTLV I, but positive results must be confirmed by another technique.

The first human retrovirus to be described, human T cell leukaemia virus I, was isolated in 1980.¹ Although most infected people remain healthy, infection with HTLV I is associated with adult T cell leukaemia² and tropical spastic paraparesis.³ HTLV I is endemic in many parts of the world, notably the islands of South West Japan² and the Caribbean basin.⁴ The first report of infection with HTLV I in the United Kingdom described six patients with adult T cell lymphoma leukaemia, all of Caribbean origin,⁵ and this association was confirmed by Greaves *et al.*,⁶ who also found antibodies to HTLV I in 5-10% of non-leukaemic Caribbean immigrants.

Although HTLV I is found in the United Kingdom, particularly among those of West Indian origin who may present with HTLV I-related disease, few laboratories offer a diagnostic service. Two commercial kits are now available which detect anti-HTLV I and these are compared with two assays in use at the Virus Reference Laboratory.

Material and methods

A total of 330 sera were examined from a variety of sources. Sera were collected from 185 women attending a breast cancer clinic in a West African country. A further 106 specimens from drug abusers were originally collected for investigation of hepatitis B infection and 39 sera were sent for investigation of HTLV I infection.

ASSAYS

Indirect immunofluorescence

This was performed as described by Hinuma *et al.*² Briefly, drops of a suspension of HTLV I infected cells were air dried on to the top row of wells of polytetrafluorethylene coated slides (C A Hendley & Co). Uninfected human lymphocytes (HT/H9 cell line) were applied to the bottom row and the cells were fixed in acetone for 10 minutes. Specimens, diluted 1/10 in phosphate buffered saline (PBS), were applied to infected and uninfected cells and incubated in a moist chamber at 37°C for one hour. The slides were washed three times in PBS for a total of 30 minutes and dried. Bound antibody to HTLV I was detected by rabbit anti-human IgG conjugated with fluorescein isothiocyanate (Wellcome Reagents) applied to each well. After incubation at 37°C in a moist chamber for one hour the slides were washed as before and examined under water immersion with a fluorescence microscope. Anti-HTLV I positive sera gave brilliant fluorescence with a granular appearance. Large aggregates were often visible in the cytoplasm.

Competitive radioimmunoassay (COMPRIA)

This was performed as described by Mortimer *et al.*⁷ for human immunodeficiency virus but with HTLV I specific reagents. Briefly, polystyrene beads were coated first with anti-HTLV I IgG and then viral antigen. The specimen was added together with a radiolabelled specific antibody and incubated overnight. If anti-HTLV I was present in the specimen, it inhibited the binding of the labelled antibody to the antigen. The per cent inhibition was calculated.

ELISA (HTLV I ELISA)

This enzyme based assay (Du Pont Ltd) consists of microtitre wells coated with virus disrupted by detergent. Diluent, specimens, and controls were added to the wells according to the manufacturer's instructions. If anti-HTLV I was present it bound to the viral antigen and was detected by the addition of goat anti-human IgG conjugated with alkaline phosphatase followed by the substrate (para nitrophenyl phosphate). A yellow colour was produced and read on a spectrophotometer.

Particle agglutination

Serodia-ATLA (Fujirebio Inc. distributed by Diamed Diagnostics Ltd) uses magenta coloured gelatin particles coated in detergent disrupted virus.⁸ Specimens were tested in microtitre plates using 0.025 ml unit volumes. Doubling dilutions of serum were made across three wells. A volume of unsensitised particles was added to the second well and a volume of virus coated particles to the third well. Positive specimens caused agglutination of the particles. Occasionally

non-specific agglutination occurred in the second well. These specimens were absorbed with unsensitised particles and the test repeated.

All specimens were examined by at least three assays and most by all four. Specimens were considered to contain anti-HTLV I if a repeatable positive result was obtained in at least three assays. Western blots (Du Pont Ltd) were prepared of four specimens which were

Table 1 Results obtained when 330 specimens were screened for anti-HTLV I

True positive n = 33	4 assays positive PA + /IF + /ELISA + PA + /IF + /ELISA + /Comp - PA + /Comp + /WB + /ELISA - /IF -	23 8 1 1
True negative n = 297	4 assays negative PA - /IF - /ELISA - IF - /ELISA - /Comp - /PA + IF - /ELISA - /WB - /PA + /Comp + PA - /ELISA - /Comp - /IF + PA - /IF - /Comp - /ELISA +	279 1 8 3 2 4

PA = particle agglutination; IF = immunofluorescence; Comp = competitive RIA; WB = Western blot.

Table 2 Comparison of assays for anti-HTLV I

	Immunofluorescence	COMPRIA	Particle agglutination	ELISA
Sensitivity (%)	96.9	92	100	97
Specificity (%)	99.3	98.9	96.3	98.6
Duration of test (hours)	4	24	4	4
Special facilities or equipment	Viral culture; fluorescence microscope	Viral culture; radioactive isotopes; counter; plate washer	None	Plate washer; spectrophotometer
Objective	No	Yes	No	Yes

Range of antibody titres to HTLV I by particle agglutination (—); ELISA (— — —); COMPRIA (· — · — ·); immunofluorescence (— · — ·).

positive by two of the four assays. Doubling dilutions of 25 positive specimens were made and the titre of antibody determined by each method.

Results

Thirty three of the 330 sera examined were anti-HTLV I positive, 31 being positive by all the assays applied (table 1). Two hundred and eighty of the remaining 297 sera were negative by all assays used, but 17 gave false positive results. The sensitivity, specificity, and technical features on the four assays are shown in table 2. All the assays performed well, but particle agglutination was the most sensitive though the least specific. Immunofluorescence was the most specific. When particle agglutination was performed as recommended by the manufacturer there was a tendency for agglutination due to strongly positive specimens to collapse inwards and the result to be misread as negative. This did not occur if the particles were diluted 1/2 in PBS.

Titres of anti-HTLV I were determined in 23 serum and two cerebrospinal fluid samples. These specimens were from seven patients with tropical spastic paraparesis, four patients with haematological disease, and 12 asymptomatic carriers. Titres measured by particle agglutination were 10–100 times higher than by the other assays (figure). Although some specimens had titres of almost 10^6 by particle agglutination no prozone effect was observed. In this small group patients with tropical spastic paraparesis generally had a higher titre of anti-HTLV I than carriers or those with haematological disease.

Discussion

There are few diagnostic facilities for HTLV I infection, but laboratories serving a population with a high proportion of those of Caribbean or African origin may wish to do diagnostic work and epidemiological studies. This study shows that a simple and sensitive agglutination test is available to them, which, however, is not very specific.

HTLV I is present in several sections of the United Kingdom population,⁹ and it has been established that HTLV I is readily transmitted by cellular blood products¹⁰ and by breast milk.¹¹ Future control of HTLV I infection may therefore require screening of some blood and avoidance of breast feeding by anti-HTLV I positive mothers. Although Serodia-ATLA is

the method of choice for this, positive results would have to be confirmed by another technique such as immunofluorescence or ELISA to exclude false positive reactions. Moreover, appropriate advice would have to be offered to subjects screened for HTLV I infection, preferably based on firmer knowledge about prognosis than is currently available. Unless the issue is dealt with sensitively, carriers are likely to be unjustifiably alarmed by learning that they carry "a leukaemia virus".

I am grateful to Dr P P Mortimer for his help in the preparation of this paper.

References

- Poiesz BJ, Ruscetti FW, Grazdar AF, Bunn PA, Minna JD, Gallo RC. 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* 1980;77:7415–19.
- Hinuma Y, Nagata K, Hanaoka M, *et al*. Adult T cell leukaemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 1981;78: 6476–80.
- Gessain A, Barin F, Vernant JC, *et al*. Antibodies to human T-lymphotropic virus type I in patients with tropical spastic paraparesis. *Lancet* 1985;ii:407–9.
- Blattner WA, Gibbs WN, Saxinger C, *et al*. Human T-cell leukaemia/lymphoma virus-associated lymphoreticular neoplasia in Jamaica. *Lancet* 1983;iii:61–4.
- Catovsky D, Greaves MF, Rose M, *et al*. Adult T cell lymphoma-leukaemia in blacks from the West Indies. *Lancet* 1982;i:639–43.
- Greaves MF, Verbi W, Tilley R, *et al*. Human T cell leukaemia virus (HTLV) in the United Kingdom. *Int J Cancer* 1984; 33:795–806.
- Mortimer PP, Jesson WJ, Vandervelde EM, Pereira MS. Prevalence of antibody to human T lymphotropic virus type III by risk group and area, United Kingdom 1978–84. *Br Med J* 1985;290:1176–8.
- Ikeda M, Fujino R, Matsui T, Yoshida T, Komada H, Imai J. A new agglutination test for serum antibodies to adult T cell leukaemia virus. *Gann* 1984;75:845–8.
- Tedder RS, Shanson DC, Jeffries DJ, *et al*. Low prevalence in the UK of HTLV I and HTLV II infection in subjects with AIDS, with extended lymphadenopathy, and at risk of AIDS. *Lancet* 1984;ii:125–7.
- Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T cell leukaemia virus by blood transfusion: seroconversion in recipients. *Vox Sang* 1984;46:245–53.
- Kinoshita K, Hino S, Amagasaki T, *et al*. Demonstration of adult T cell leukaemia virus antigen in milk from three sero-positive mothers. *Gann* 1984;75:103–5.

Requests for reprints to: Dr Philippa M B White, Associate, Virus Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, England.