Serologic testing for human T-lymphotropic virus-3 and -4

Human T-lymphotropic viruses (HTLVs) are a diverse group of deltaretroviruses that until recently included only two unique groups, HTLV-1 and HTLV-2.[1,2] Both HTLV-1 and HTLV-2 are spread by sexual transmission, from mother to child, and by exposure to infective blood through transfusions and injection drug use.[1,2] HTLV-1 and HTLV-2 are pathogenic—although in less than 5 percent of infected persons—causing lymphoproliferative and inflammatory diseases. Thus, blood donated for transfusion is tested for potential HTLV infectivity in Japan and other countries in Asia, the United States, and Europe. Recently, two new, highly divergent HTLVs, designated HTLV-3 and HTLV-4, were identified in persons in Cameroon with exposure to primates.[3,4] HTLV-3 was found in two persons and HTLV-4 was found in a single individual.[3,4] HTLV-3 is genetically similar to simian T-lymphotropic virus type 3 (STLV-3), whereas HTLV-4 is the only member of a novel deltaretrovirus group that is equidistant from HTLV-1/STLV-1, HTLV-2/STLV-2, and HTLV-3/STLV-3 and does not have a known STLV counterpart.[3,4] HTLV groups are currently classified with Arabic numerals following the guidelines of the International Committee on Taxonomy of Viruses.[5] The deltaretroviruses HTLV-3 and HTLV-4 should not be confused with HTLV-III and HTLV-IV because the latter are the original names given to the lentiviruses human immunodeficiency virus (HIV)-1 and HIV-2 because they were isolated from infected human T cells.[6]

The identification of these new divergent HTLVs raises questions whether they can be detected by current serologic assays for HTLV antibodies, which are licensed by the Food and Drug Administration (FDA) for testing donated blood in the United States. Anti-HTLV-3 and anti-HTLV-4 antibodies were detected in plasma by screening with an HTLV-1 and -2 enzyme immunoassay (EIA; Vironostika HTLV-1/2 microELISA system, Organon Teknika, Durham, NC) or an HTLV-1 immunofluorescent assay (Table 1).[3,4] It is not known, however, whether other blood screening assays can detect antibodies to these novel HTLVs.[3,4] In addition, supplementary testing showed variable HTLV serotyping results for these African samples with an HTLV Western blot (HTLV WB 2.4, Genelabs Diagnostics, Singapore), with the HTLV-3–infected samples being typed as indeterminate[4] or HTLV-1–like[3] and the HTLV-4–infected sample giving an HTLV-2–like profile.[3] These WB results may help to explain why these viruses have not been previously identified and suggest that additional HTLV-3 and HTLV-4 may be circulating undetected, incorrectly identified as either HTLV-1 or HTLV-2.

To evaluate whether other HTLV serologic assays can detect HTLV-3 and HTLV-4 and differentiate these viruses from HTLV-1 and HTLV-2, we tested plasma samples available from two of the three infected persons from Cameroon[3] with an FDA-licensed HTLV EIA (HTLV-1 and -2 EIA, Abbott Diagnostics, North Chicago, IL) and a supplementary research-based line immunoassay (LIA; INNO-LIA, Innogenetics, Ghent, Belgium). Like the FDA-licensed Vironostika EIA, the Abbott EIA uses an HTLV-1 and -2 viral lysate but does not include a recombinant HTLV-1 p21 envelope (Env) protein. INNO-LIA uses recombinant HTLV-1 and -2 generic and type-specific proteins and synthetic peptides placed in lines on a nylon membrane for the detection and discrimination of HTLV-1 and -2. The research-based HTLV WB 2.4 employs an HTLV-1 lysate and HTLV-1 and HTLV-2 type-specific peptides for serotyping.

The HTLV-3 and HTLV-4 samples both reacted in the Abbott EIA with OD-to-cutoff ratios ranging from 1.7 to 3.6, respectively. The HTLV-3 sample gave a weak positive, but untypeable INNO-LIA profile, whereas the HTLV-4 specimen showed an HTLV-2–like INNO-LIA profile (Table 1). Plasma from the second HTLV-3–infected person (Pyl43) has been reported to have an HTLV-2–like INNO-LIA profile but is indeterminate in the HTLV WB 2.4 test.[4]

Both samples were detected by EIA testing, suggesting that the two new HTLV variants do not pose a diagnostic challenge for these two FDA-licensed HTLV EIAs used to test donated blood in the United States. The overall sensitivity of both EIAs for identifying HTLV-3– and HTLV-4–infected samples is unknown, however, and requires testing of a larger number of specimens from HTLV-3– and HTLV-4–infected persons. The WB and INNO-LIA results demonstrate the need to design specific antibody assays to differentiate HTLV-3 and HTLV-4 from HTLV-1 and HTLV-2. The combination of accurate screening and improved supplementary tests to identify and differentiate the four HTLV groups will facilitate determination of the prevalence and risks for secondary transmission of these new viruses.

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TABLE 1. Results of testing samples containing HTLV-3 and HTLV-4 with screening and confirmatory assays for HTLV-1 and HTLV-2

Virus*	Vironostika†	Abbott†	IFA‡	WB 2.4§	Interpretation	INNO-LIA§	Interpretation
HTLV-3 (2026ND)	3.45	1.78	ND	Weak rgp21 Env, p19 and p24 Gag, and HTLV-1 type–specific peptide	HTLV-1–like	Weak generic p24 Gag and gp21 Env	Indeterminate
HTLV-3 (Pyl43)	ND	ND	Positive	p19 Gag, HTLV-1 type–specific peptide	Indeterminate	Weak generic p19 Gag, strong generic Env gp46, HTLV-2 type–specific gp46	HTLV-2–like
HTLV-4 (1863LE)	4.26	3.62	ND	Weak rgp21 and gp46 Env, p19 Gag, weak p24 Gag, weak HTLV-2 type–specific peptide	HTLV-2–like	Generic p19/p24 Gag and gp21/gp46 Env, HTLV-2 gp46 Env > HTLV-1	HTLV-2–like

* Virus strains are shown in parentheses.[3,4]
† OD/cutoff ratios. ND = not done, specimen or assay not available for evaluation.
‡ IFA = immunofluorescence assay with an HTLV-1-infected cell line.[4]
§ Env, envelope; rgp, recombinant glycoprotein in Env; Gag, group-specific antigen.

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Inactivation of parvovirus B19 and model viruses in factor VIII by dry heat treatment at 80°C

The viral safety of plasma-derived products such as factor (F)VIII is achieved by a combination of donor selection, plasma screening, and the inclusion of viral inactivation and removal steps in the manufacturing process. Enveloped viruses such as human immunodeficiency virus, hepatitis B virus, and hepatitis C virus are effectively controlled by such procedures. Nonenveloped viruses, however, are generally more resistant to inactivation. Hepatitis A (HAV) and human parvovirus B19 are two nonenveloped viruses that can be transmitted by plasma products. Because B19 itself has proved difficult to grow in cell culture, inactivation studies have routinely been carried out with animal parvoviruses such as canine parvovirus (CPV), bovine parvovirus (BPV), porcine parvovirus (PPV)