

Kaposi's Sarcoma-Associated Herpesvirus Serology in Europe and Uganda: Multicentre Study With Multiple and Novel Assays

Octavian Schatz,^{1*} Paolo Monini,² Roberto Bugarini,³ Frank Neipel,⁴ Thomas F. Schulz,⁵ Massimo Andreoni,⁶ Peter Erb,⁷ Maren Eggers,⁸ Jürgen Haas,⁹ Stefano Buttò,² Matthew Lukwiya,¹⁰ Johannes R. Bogner,¹ Svenja Yaguboglu,⁴ Julie Sheldon,⁵ Loredana Sarmati,⁶ Frank Detlef Goebel,¹ Renate Hintermaier,¹ Gisela Enders,⁸ Nicolas Regamey,⁷ Marion Wernli,⁷ Michael Stürzl,¹¹ Giovanni Rezza,³ and Barbara Ensoli^{2**}

¹*Infektionslabor, Med. Poliklinik, Ludwig-Maximilians-Universität, Munich, Germany*

²*Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy*

³*Laboratory of Epidemiology and Biostatistics, Istituto Superiore di Sanità, Rome, Italy*

⁴*Institute for Clinical and Medical Virology, University of Erlangen-Nürnberg, Erlangen, Germany*

⁵*Department of Medical Microbiology, The University of Liverpool, Liverpool, United Kingdom*

⁶*Department of Infectious Diseases, University of Tor Vergata, Rome, Italy*

⁷*Institute for Medical Microbiology, University of Basel, Basel, Switzerland*

⁸*Medizinisch-Diagnostisches Gemeinschaftslabor, Institute for Virology, Infectiology and Epidemiology, Stuttgart, Germany*

⁹*Max von Pettenkofer-Institute, Genzentrum, Munich, Germany*

¹⁰*St. Mary's Hospital Lacor, Gulu, Uganda*

¹¹*GSF-National Research Center for Environment and Health, Institute of Molecular Virology, Neuherberg, Germany*

A multicentre study was undertaken to define novel assays with increased inter-assay concordance, sensitivity, specificity and predictive value for serological diagnosis of human herpesvirus type 8 (HHV-8) infection. A total of 562 sera from European and Ugandan human immunodeficiency virus (HIV)-infected or uninfected individuals with or without Kaposi's sarcoma (KS) and blood donors were examined under code by 18 different assays in seven European laboratories. Sera from KS patients and all non-KS sera found positive by at least 70%, 80%, or 90% of the assays were considered "true positive." The validity of the assays was then evaluated by univariate logistic regression analysis. Two immunofluorescence assays (IFA) for detection of antibodies against HHV-8 lytic (Rlyt) or latent (LLANA) antigens and two enzyme-linked-immunosorbent assays (ELISA) (M2, EK8.1) for detection of antibodies against HHV-8 structural proteins were found to be highly concordant, specific, and sensitive, with odds ratios that indicated a high predictive value. When used together, the two IFA (Rlyt-LLANA) showed the best combination of sensitivity (89.1%) and specificity (94.9%). The performance of these assays indicate that they may be used for the clinical management of individuals at risk of developing HHV-8 associated tumours such as allograft recipients. **J. Med. Virol. 65:123–132, 2001.** © 2001 Wiley-Liss, Inc.

KEY WORDS: organ transplantation; risk management; serodiagnosis

INTRODUCTION

Human herpesvirus-8 (HHV-8) [Chang et al., 1994] is a γ -herpesvirus associated with the development of Kaposi's sarcoma (KS), a proliferative disease of vascular origin particularly frequent in individuals infected with the human immunodeficiency virus (HIV),

Grant sponsor: Istituto Superiore di Sanità "III National Research Programme on AIDS," Italian Ministry of Health; Grant sponsor: "III National Research Programme on AIDS"-Concerted Action "Epidemiology," Italian Ministry of Health; "Uganda AIDS Project," Italian Ministry of Health; Grant sponsor: Associazione Italiana per la Ricerca sul Cancro (AIRC); Grant sponsor: Friedrich-Baur-Stiftung; Grant number: RK 8233049; Grant sponsor: NHS Biomedical Research Frame; Grant number: RDO/22/09; Grant sponsor: Swiss National Science Funds; Grant number: 31-064233.00; Grant sponsor: Deutsche Forschungsgemeinschaft SFB 466: "Lymphoproliferation and Viral Immunodeficiency"; Grant sponsor: European Concerted Action "Pathogenesis of AIDS-KS".

*Correspondence to: Octavian Schatz, DIAVIR, Ltd., Steinbergstrasse 30, D-85250 Altomuenster, Germany. E-mail: drschatzo@aol.com

**Correspondence to: Barbara Ensoli, Laboratory of Virology, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161, Rome, Italy. E-mail: ensoli@iss.it

Accepted 23 October 2000

elderly people from Mediterranean countries, African populations, and kidney allograft recipients [Chang et al., 1994; Ensoli and Stürzl, 1998; Schulz, 1999]. HHV-8 DNA is found invariably in KS lesions [Moore et al., 1996; Dupon et al., 1997; Knowles and Cesarman, 1997], and several lines of evidence suggest that HHV-8 infection is required for KS development. In fact, HHV-8 seroprevalence is high in population groups or geographical areas with high incidence of KS [Kedes et al., 1996; Simpson et al., 1996; Gao et al., 1996b; Calabrò et al., 1998; Olsen et al., 1998], and HHV-8 infection in individuals at risk for KS is predictive of KS development [Whitby et al., 1995; Gao et al., 1996a; Renwick et al., 1998; Farge et al., 1999; Rezza et al., 1999]. In addition, HHV-8 is present in a latent form in KS spindle cells that are considered to be the tumour cells of KS [Boshoff et al., 1995; Stürzl et al., 1997; Ensoli and Stürzl, 1998; Schulz, 1999]. Moreover, KS develops and progresses with an intermittent or persistent HHV-8 viraemia [Whitby et al., 1995; Decker et al., 1996; Harrington et al., 1996; Monini et al., 1999] and recent data indicate that infection is reactivated by the same inflammatory cytokines increased in individuals with KS or at high risk of developing KS [Monini et al., 1999].

HHV-8 infection is also associated with primary effusion lymphomas (PEL), a rare form of lymphoma without clear nodal origin arising almost exclusively in patients with AIDS [Cesarman et al., 1995; Gaidano et al., 1996], and multicentric Castleman's disease, a lymphoproliferative disease often occurring in HIV positive or negative individuals with KS [Soulier et al., 1995; Grandadam et al., 1997]. HHV-8 DNA is invariably detected in PEL and PEL-derived cell lines, whereas it is absent in other AIDS-associated lymphomas [Gaidano et al., 1996]. In addition, a high HHV-8 DNA load in peripheral blood mononuclear cells from patients with multicentric Castleman's disease correlates with the exacerbation of disease symptoms [Grandadam et al., 1997]. Thus, HHV-8 infection probably plays a causative role also in these lymphoproliferative disorders.

In the general population, HHV-8 seroprevalence shows marked geographical variations, with a relatively wide distribution in Southern Europe as compared to Northern Europe and North America, and with the highest prevalence in African countries [Kedes et al., 1996; Simpson et al., 1996; Gao et al., 1996b; Calabrò et al., 1998; Olsen et al., 1998]. In different socio-economic settings HHV-8 seroconversion occurs at different ages, and the virus is likely transmitted by different modes [Kedes et al., 1996; Blauvelt et al., 1997; Bourboulia et al., 1998; Calabrò et al., 1998; Olsen et al., 1998; Regamey et al., 1998a; Andreoni et al., 1999; Schulz, 1999; Sitas et al., 1999; Smith et al., 1999]. In the developed countries, HHV-8 seroconversion is observed in the adult age and the virus is transmitted most likely by sexual contact [Kedes et al., 1996; Blauvelt et al., 1997; Regamey et al., 1998a; Calabrò et al., 1999; Smith et al., 1999], whereas the

frequent seroconversions observed in children from African countries are probably due to virus transmission by saliva or mother-to-child transmission [Bourboulia et al., 1998; Andreoni et al., 1999; Schulz, 1999; Sitas et al., 1999].

The determination of the HHV-8 serostatus is important for surveillance and prevention of HHV-8-associated diseases, particularly in organ transplantation and in the clinical management of HIV infection. In fact, kidney or heart allograft recipients who are infected by HHV-8 due to organ transplantation have a high risk of KS or PEL development [Regamey et al., 1998b; Dotti et al., 1999; Farge et al., 1999] probably due to HHV-8 reactivation and active replication [Hudnall et al., 1998]. The risk of KS may be exceedingly high when the organ donor and recipient are both HHV-8 positive [Parravicini et al., 1999]. In addition, the HHV-8 serostatus appears to be critical for people exposed to HIV infection, and the risk of KS is particularly high in individuals that are infected by HHV-8 after HIV seroconversion [Renwick et al., 1998; Rezza et al., 1999]. So far, however, the design of an algorithm for serological diagnosis of HHV-8 infection has been hampered by the poor concordance of the available serological assays [Rabkin et al., 1998]. The results are described of a multicentre study aimed at defining novel or improved HHV-8 serological assays or combination of assays with increased concordance, sensitivity, specificity, and predictive value.

MATERIALS AND METHODS

Serum Panels

Five serum panels containing a total of 562 individual sera were generated and coded by the laboratories participating to the study. Panel Munich 1 consisted of 100 samples (23 sera from AIDS-KS patients, 1 PEL patient, 36 sera from HIV-1 infected individuals without clinical signs of KS (HIV positive - KS negative), 2 patients with lymphoma, and 38 sera from healthy individuals). The HIV-1 infected patients were asymptomatic (33 subjects) or full-blown AIDS (3 patients); 30 out of these subjects were males, the majority of whom were infected by homosexual contact. Panel Munich 2 consisted of 173 serum samples from 12 patients with AIDS-KS, 80 HIV positive-KS negative individuals (8 with AIDS and 72 asymptomatic; 65 were males, the majority of whom experienced homosexual practices), and 81 healthy individuals. The Basel panel contained 25 sera from AIDS-KS patients, 5 from HIV positive-KS negative individuals, 33 from individuals seeking HIV counselling who experienced unprotected sexual contacts or due to anxiety, and 37 from blood donors. The Rome panel contained 87 sera from Italian blood donors. The Uganda panel contained a total of 102 sera from HIV positive or HIV negative patients attending various clinics at the St. Mary's Lacor-Hospital in Gulu, North Uganda. Nine out of 102 sera were from AIDS patients, 44 from pregnant women (one of whom was seropositive for HIV-1), 34 from patients with various

diseases (12 HIV positive), and 15 with tuberculosis (3 of which HIV positive).

After the results of the serological assays were reported, the codes were broken and the data analysed. Diagnosis of HIV infection was confirmed by testing all sera for anti-HIV-1 antibodies, except for the Munich 2 panel the sera of which had been screened for HIV-1.

Serological Assays

Six immunofluorescence assays (IFA) (LLANA, Rlyt, Rlat, SB1, and SBio) and 8 enzyme-linked-immunosorbent assays (ELISA) (B65, EK8.1, EK12, E65, L65, L73, M1, and M2) were used. The first letter of the above acronyms identifies the city in which the test was developed or performed (B = Basel, E = Erlangen, L = Liverpool, M = Munich, R = Rome, S = Stuttgart).

The IFA included assays for the detection of antibodies against both HHV-8 latent (LLANA, Rlat, SB1, and SBio) or lytic (Rlyt) antigens. M1 and M2 are two versions of a novel ELISA based on a mixture of peptides from the HHV-8 open reading frames (ORF) 20, 65, and K8.1A. The other ELISAs (B65, EK8.1, EK12, E65, L65, and L73) are based on recombinant proteins from single HHV-8 ORFs. The numbers in the acronyms identify the respective HHV-8 ORF. Additional ELISAs (for a total of 12 ELISAs) were undertaken with peptides specific for a single HHV-8 ORF selected from the peptide mixture used for M1 or M2 ELISA.

LLANA (Liverpool Latent Nuclear Antigen IFA)

This immune fluorescence assay is based on the PEL cell line BCP-1 cells and was carried out as described previously [Simpson et al., 1996]. Briefly, BCP-1 cells were washed in phosphate buffered saline (PBS), smeared on microscope multiwell slides, fixed in 4% paraformaldehyde, permeabilised in 0.2% Triton X-1000, blocked in 100 mM glycine, and followed by 10% foetal calf serum (FCS). Patient sera were diluted 1:50 in PBS containing 2% FCS, added to slides for 1 hour, followed by three washes in PBS, and incubated with a FITC-conjugated anti-human IgG antibody (DAKO).

SB1 and Sbio (Stuttgart BCBL-1 and Stuttgart Biotrin IFAs)

The SB1 IFA was carried out with the PEL cell line (BCBL-1) (provided by R. Renne, San Francisco, CA) [Renne et al., 1996]. A suspension of BCBL-1 cells was spotted in 21-well slides and cells were air-dried and fixed with acetone. For IFA, slides were first incubated with serum samples diluted to 1:50 and 1:100 for 30 minutes at 37°C, followed by incubation with fluorescein-conjugated goat anti-human antibody as the secondary antibody. Slides were scored independently by two operators. Samples with titres of > 1:100 were considered positive. Serial dilutions of all positive

serum samples were independently tested a second time. SBio is a commercial kit (Biotrin, Ltd., Dublin, Ireland) and was used according to the instructions of the manufacturer.

Rlyt and Rlat (Rome Lytic and Latent Antigens IFAs)

These assays are based on BCBL-1 cells and were carried out as described [Andreoni et al., 1999, Rezza et al., 1999]. Briefly, to detect anti-lytic antigen antibodies tetradecanoylphorbol acetate (TPA)-induced BCBL-1 cells [obtained from Dr. M. McGrath and Dr. D. Ganem through the AIDS Research and Reference Reagent Program, Division of National Institute of AIDS, Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH)] were smeared on slides and fixed with a methanol/acetone (1:1; vol/vol) solution at -20°C for 10 min, whereas IFA to detect anti-latent antigen antibodies was performed with nuclei of BCBL-1 cells isolated as previously described [Andreoni et al., 1999]. Fixed smears were pre-blocked by incubation with PBS supplemented with 3% FCS for 30 minutes in a humidified chamber and then incubated successively in two steps of 45 minutes each at 37°C with the test serum diluted 1:10 with PBS supplemented with 1% glycine and 2% FCS or with goat fluorescein isothiocyanate-conjugated anti-human antibodies. Titrations were carried out by 4-fold serial dilutions. All microscopic examinations were blinded.

EK8.1, EK.12, E65 (Erlangen orfK8.1, orfK12, or orf65 ELISAs)

For EK8.1, a polypeptide comprising amino acids 27–196 of HHV-8 K8.1A (= K8.1beta) spliced mRNA [Raab et al., 1998] including an N-terminal histidine-tag (MRGSHHHHHGS), was expressed in *Escherichia coli* (*E. coli*) and the recombinant protein was purified by Ni-affinity chromatography. For E65 and EK12, the complete coding regions from ORF65 and K12/KaposinA were amplified from HHV-8 genomic DNA, expressed as glutathione S-transferase (GST)-fusion proteins. The fusion proteins were purified as described previously [Lang et al., 1999]. Polystyrene microplates (96-wells) were coated with purified antigens (1 µg/ml) or blocking buffer (PBS, 2% FCS) according to standard procedures. Sera were diluted 1:50, added to coated wells, and incubated for 60 minutes at 37°C. Wells were washed four times with blocking buffer before the addition of a goat anti-human immunoglobulin coupled to horseradish peroxidase (DAKO). After incubation at 37°C for 30 minutes, wells were washed four times with blocking buffer and incubated for 30 minutes at 22°C with O-phenylenediamine (Sigma Chemical Co., Buchs, Switzerland) supplemented with 30% H₂O₂ (16.6 µl/10 ml), followed by incubation for 15 minutes at 37°C. The reaction was stopped with 1 N sulphuric acid. The cut-off for EK8.1 was determined on 177 human sera that were found to be non-reactive by lytic IFA on

BCBL-1 cells and Western blot for HHV-8 proteins K8.1, ORF73, K12, and ORF65. Cut-off values for K12 and ORF65 ELISA were calculated as described elsewhere [Lang et al., 1999].

B65 (Basel orf65 ELISA)

Recombinant proteins corresponding to the amino acids 86-170 of the HHV8 ORF65 were expressed in M14 bacteria and purified by affinity chromatography on Ni-NTA resin (Qiagen, Basel, Switzerland). The assay was carried out as described previously [Regamey et al., 1998a,b]. Briefly, microplates (Greiner, Frickenhausen, Germany) were coated overnight with 100 μ l of purified protein (2 μ g/ml) in 0.1 N NaHCO₃, pH 8.5, at 4°C. The plates were washed with PBS containing 0.1% Tween 20 (PBS-T) and saturated with 5% dried skimmed milk in PBS-T (blocking buffer). Patient sera were diluted 1:100. For serum IgG detection, a peroxidase conjugated goat anti-human IgG (Sigma Chemical Co., Buchs, Switzerland) (dilution 1:2,000) was used as described by the manufacturer. The mean plus five standard deviations of the optical density values from five blood donor sera was taken as "cut-off" value for each plate. Two reactive sera of patients with KS were used as positive controls on each plate.

L65, L73 (Liverpool orf65 and orf73 ELISAs)

Recombinant orf65 and orf73 derived proteins were expressed in *E. coli* using the pQE42 vector (Quiagen, Hilden, Germany); the assays were carried out as described previously [Rainbow et al., 1997; Simpson et al., 1996]. Briefly, microplates (Dynatech Immulon 4) were coated for 15 hours with recombinant purified proteins dissolved in 100 mM NaHCO₃, pH 8.5. After blocking plates with PBS containing 0.1% Tween 20 and 5% MarvelTM (PBS-TM), sera were diluted 1:100 in PBS-TM, added to plates, and antibody binding detected with alkaline phosphatase conjugated goat anti-human IgG (Seralab, Crawley Down, Sussex, UK) and nitrophenyl phosphate (100 μ g/ml).

M1 and M2 (Munich peptide mix 1 and 2 ELISAs)

A library of 300 biotinylated HHV8-specific peptides was prepared by selection at random of decamer sequences from a collection of different HHV8 ORF that did not show significant homology (≥ 3 uninterrupted identical residues out of 10) to sequences of known herpesviruses. The antigenicity index of the remaining peptides was then calculated and those with the highest index were selected. The resulting library was screened for differential reactivity with sera pools from KS patients and from healthy blood donors, respectively. Immunogenic peptides which displayed at least 10-fold higher reactivity with the KS pool as compared to the donor pool were examined subsequently with a panel of individual sera. Those peptides

that showed only weak selectivity between KS and donor sera were excluded; the others were mixed in equimolar ratios and bound to streptavidin-coated microplates (Meltek Scientific, Feltham, UK, or Microcoat, Bernried, Germany). The first version (M1) of this peptide ELISA contains a set of four peptides (65A, 65X, 20A, and K8.1A, derived from the corresponding open reading frames); the second version (M2) contains a set of two peptides (65X and K8.1A) with a higher purity (>95%). The sequence of the peptides used in these assays are MSNFKVRDPV from nucleotide (nt) 112443 to 112414 of the HHV-8 genome for 65A, ARKPPSGKKK (nt 111960–111931) for 65X; MYEV-FTDFPV (nt 35573–35544) for 20A, and PTYRSHLG-FWQE (nt 75999–76034) for K8.1A (HHV-8 nucleotide sequence as in GenBank accession number U75698). Test sera were used at a 1:100 to 1:200 dilution, detection was performed with a 1:10,000 dilution of rabbit anti-human IgG-(Heavy plus light chain) peroxidase conjugate (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and 2,2'-Azino-bis-(3-ethylbenzthiazolin-6-sulphonic acid) (ABTS) as substrate (Boehringer Mannheim Biochemicals, Indianapolis, IN or Devitron, Castrop-Rauxel). Individual blanks (without peptides) were used to identify false positives.

Statistical Analysis

To evaluate the concordance among assays the observed agreement and the *k* statistic were calculated. The observed agreement (which was used to assess the average concordance between a given assay and all the others) does not take into account the expected agreement [Maclure and Willet, 1987]. The *k* statistic was used to compare pairs of assays providing information on the agreement beyond chance [Fleiss, 1981]. A value of 1.0 was considered as "perfect agreement" and 0 as "no agreement beyond chance"; values between .40 and .75 were considered to represent a "fair to good agreement beyond chance" values <20 were considered as a "weak agreement" [Fleiss, 1981].

In the absence of a gold standard, sensitivity [= true positives/(true positives + false negatives)] and specificity [= true negatives/(true negatives + false positives)] were calculated taking as "true positive" all sera from patients with KS and those sera from non-KS individuals that scored positive with at least six (70%), 7 (80%), or 8 (90%) of the assays used to screen all the five serum panels (see below).

An univariate logistic regression model was performed to evaluate the validity of a given test. The model permits to calculate the OR for any specific assay based on the formula $OR = [(PPV)/(1-PPV)]/[(1-NPV)/(NPV)]$, where PPV (positive predictive value) is the probability that a positive result identifies a true HHV-8 positive serostatus and NPV (negative predictive value) is the probability that a negative result identifies a true HHV-8 negative serostatus. Considering "x" as the response of the assay (HHV-8 positive, x = 1; HHV-8 negative, x = 0), and $\pi(x)$ the probability of being true

HHV-8 positive, the OR is given by the formula $\{\pi(1)/[1-\pi(1)]\}/\{\pi(0)/[1-\pi(0)]\}$, where the OR represents the risk of any individual with a positive test to be infected as compared to the risk of individuals with a negative test. This model, which accounted for the correlation among repeated measurements in the same individuals, allows evaluation of the validity (sensitivity, specificity, and predictive value) of a given test independently of the true prevalence of HHV-8 infection in the population [Choi, 1997]. Calculations to determine sensitivity, specificity, and OR were repeated using the three different proportions of sera considered to be true positives according to the above assumptions (i.e., true positive all KS sera plus positive non-KS sera with at least 70%, 80%, or 90% of the nine assays used to screen all the five serum panels).

RESULTS

Characteristics of the Study Population

Five serum panels of 562 serum samples, (460 from Caucasian and 102 from Ugandan individuals) were evaluated. Of the 460 Caucasian serum samples, 373 were from individuals from Germany and Switzerland. Of these, 60 were from patients with AIDS-KS, 121 from KS-free HIV positive individuals, 159 from HIV

negative healthy individuals not at risk for HIV infection, and 33 from HIV negative individuals seeking HIV counselling. The remaining 87 Caucasian sera were from Italian blood donors. The Ugandan sera were from 44 pregnant women (of whom 1 HIV positive), 9 AIDS cases, 15 patients with pulmonary tuberculosis (of whom 3 were HIV positive), and 34 outpatients (of whom 12 were HIV positive).

Assay Concordance and HHV-8 Seroprevalence in the Population Groups

The serum samples were screened by eighteen different assays; however, only 9 assays were used to screen all the five serum panels. With these nine assays, the positivity for HHV-8 antibodies in the Caucasian sera ranged from 14.2% to 34.4% (Fig. 1A) and showed the highest prevalence in the KS group (40% to 96.8%; Fig. 1B), intermediate levels in the HIV positive-KS negative group (8.3% to 42.1%; Fig. 1C), and the lowest prevalence in the normal donor group (0.6% to 11.9%; Fig. 1D). As compared with the Caucasian sera (Fig. 1A), the Uganda population group taken as a whole showed a significantly wider range of HHV-8 seroprevalence by the various assays (18.8% to 69.3%; data not shown).

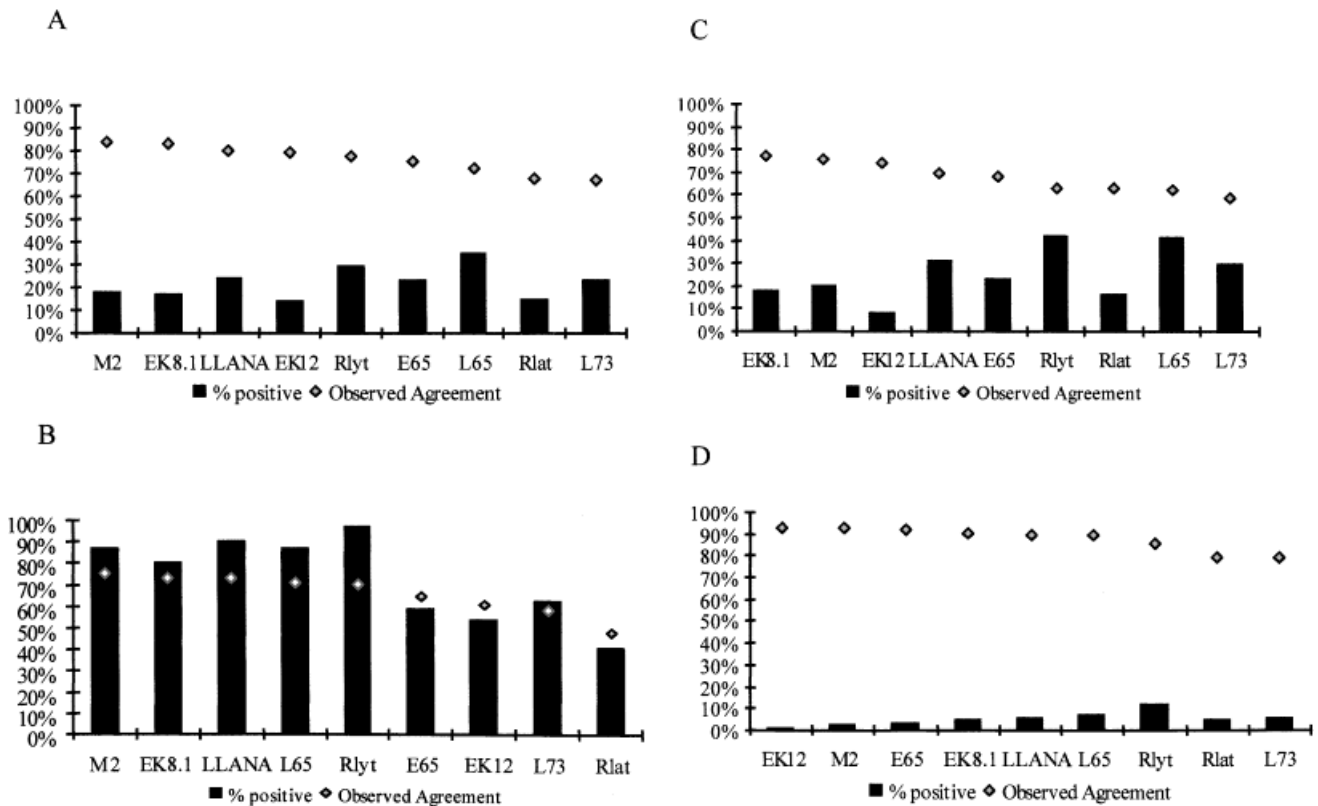


Fig. 1. Prevalence of human herpesvirus type 8 (HHV-8) antibodies and average observed agreement determined with the nine assays used to test the all five serum panels. The analysis refers to the Caucasian serum samples. Prevalence (histograms) and average observed agreement (solid triangles) for (A) 460 human immunodeficiency virus type 1 (HIV) positive or HIV negative individuals without Kaposi's sarcoma (KS), (B) 60 patients with AIDS-KS, (C) 121 HIV positive individuals without KS, and (D) 87 Italian blood donors are shown.

TABLE I. Numbers of Concordant Positive Samples (*k* Statistics in Parentheses) in Pairwise Comparisons of Human Herpesvirus Type 8 (HHV-8) Antibody Tests on 460 Caucasian Subjects[†]

Positivity		M2	L65	L73	LLANA	EK12	E65	EK8.1	Rlyt
82/459	M2	—							
158/459	L65	69 (0.425 [*])	—						
105/459	L73	50 (0.416 [*])	74 (0.387 [*])	—					
108/457	LLANA	66 (0.621 [*])	75 (0.389 [*])	63 (0.473 [*])	—				
65/459	EK12	36 (0.393 [*])	40 (0.153 [*])	34 (0.264 [*])	37 (0.300 [*])	—			
105/459	E65	44 (0.335 [*])	56 (0.195 [*])	42 (0.222 [*])	50 (0.313 [*])	45 (0.423 [*])	—		
77/459	EK8.1	65 (0.779 [*])	61 (0.354 [*])	47 (0.397 [*])	62 (0.586 [*])	36 (0.417 [*])	45 (0.370 [*])	—	
132/457	Rlyt	71 (0.560 [*])	82 (0.363 [*])	56 (0.293 [*])	77 (0.512 [*])	42 (0.268 [*])	55 (0.276 [*])	65 (0.509 [*])	—
66/457	Rlat	34 (0.354 [*])	42 (0.171 [*])	31 (0.222 [*])	34 (0.244 [*])	23 (0.241 [*])	27 (0.157 [*])	32 (0.344 [*])	56 (0.446 [*])

[†]Concordance was evaluated by the agreement beyond chance for the 9 assays that were used to screen all the 5 panels. The sera from Uganda were not included in the analysis. The *k* statistic values are significantly higher as compared to those from previous work.³²

^{*}Significant 95% ($P < 0.05$).

Concordance was evaluated only for the nine assays that were used to screen all the five panels and was first determined by the agreement beyond chance that evaluates the concordance of positive scores [Fleiss, 1981]. All the nine assays showed a significant agreement beyond chance with the 460 Caucasian sera, as evaluated by the *k* statistic values that were determined for each possible pair of assays (Table I). In particular, although the level of agreement showed a wide variation, the *k* statistic values were significantly higher as compared to those from a previous study [Rabkin et al., 1998] (Table I). The best pairwise agreement for the Caucasian group (*k* values between .560 to .779) was found between the Munich peptide mix ELISA and three other assays, namely, the Erlangen orfK8.1 ELISA, the Liverpool latent nuclear antigen IFA, and the Rome lytic antigen IFA. High *k* statistic values were found also between the Rome lytic antigen and the Liverpool latent nuclear antigen IFAs and the Erlangen orfK8.1 ELISA (*k* value between .509 to .586) (Table I). With this analysis, the KS group showed a worse agreement beyond chance and smaller *k* statistic values as compared to the whole Caucasian population group (data not shown). This, however, was anticipated, since the *k* statistic is corrected for the chance that positive scores are concordant due to high seroprevalence values (expected agreement) [Fleiss, 1981].

The concordance of the nine assays was then evaluated by determining the observed agreement that takes into account both negative and positive scores and it is calculated independently from the expected agreement [Maclure and Willet, 1987]. With this analysis, the concordance of the nine assays was generally good, ranging between 67.1 and 83.6% in the total Caucasian population (Fig. 1A), between 47.5 and 75.4% in the KS group (Fig. 1B), 59.0 and 77.2% in the HIV positive-KS negative group (Fig. 1C), and 79.3 and 93.1% in the Italian blood donor group (Fig. 1D), respectively.

The analysis of sera from Uganda showed a significantly lower concordance either by observed agreement or agreement beyond chance (only 21.1% of *k* statistics were significant) (data not shown). Nevertheless, a fair-good agreement was observed between the Munich peptide mix ELISAs (M1 and M2), and an

acceptable level of agreement for other five pairs of assays, including the Liverpool latent nuclear antigen IFA and the Liverpool orf73 ELISA (*k* = 361) and the Erlangen orfK8.1 ELISA and the Rome lytic antigen IFA (*k* = 341).

Estimates of Assay Sensitivity, Specificity, and Predictive Value

The sensitivity and specificity of the assays were next evaluated with the Caucasian sera. These parameters can be determined only upon the identification of the individuals that are “certainly” infected. A “gold standard” methodology for the identification of these individuals is not yet available for HHV-8; however, it is possible to assume that all KS patients are infected because virtually all KS lesions are HHV-8 positive by the polymerase chain reaction. In addition, the improved concordance of the nine assays used to screen all the five serum panels suggested that the positive serum samples by the majority of the nine assays consisted of true positive sera. On the basis of these assumptions, estimates of the sensitivity and specificity of the assays were obtained considering that the true positive sera included those from KS patients plus the samples that resulted positive with at least 70%, 80%, or 90% of the nine assays, respectively. Owing to the poor concordance obtained with sera from Africa, the Uganda panel was not included in this analysis.

The results of this analysis are described in Figure 2A, B. The most sensitive assay under all the above assumptions (proportion of positive tests greater than 70%, 80%, or 90%) was the IFA Rlyt (sensitivity = 97.1%), which also showed a relatively good specificity (83.2%). The IFA LLANA showed fairly high sensitivity and specificity values (90% and 87.1%, respectively). The most specific assays were the Erlangen orfK8.1 and the Munich peptide mix ELISAs (specificity = 94.9% and 94.3%, respectively), which also showed relatively high sensitivity values (81.4% and 85.7%, respectively).

To evaluate the balance between the sensitivity and specificity characteristics of the various assays, the results of the serological tests were analysed under the above assumptions by a univariate logistic regression

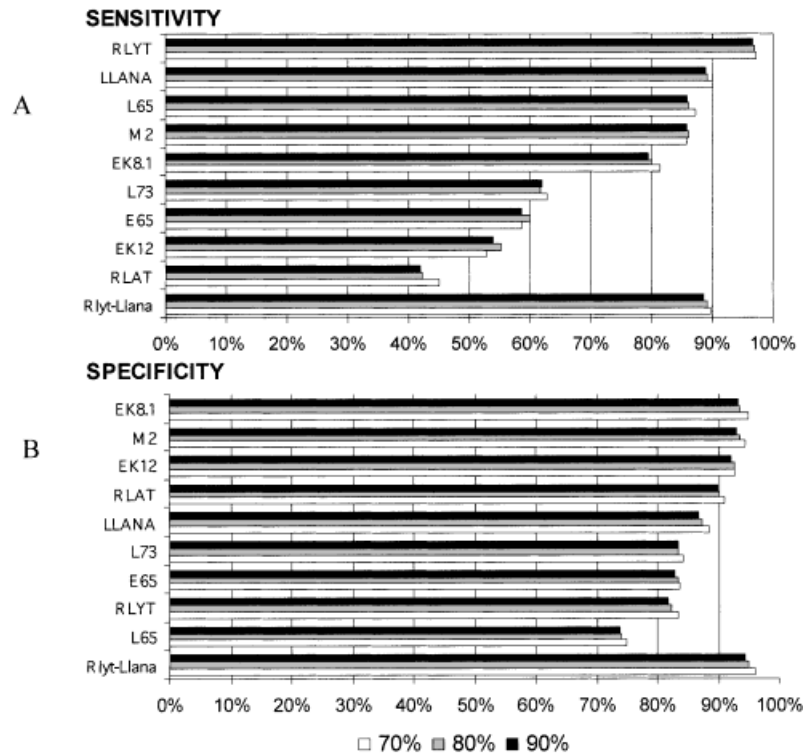


Fig. 2. (A) sensitivity and (B) specificity of the nine assays used to test the all five serum panels. Sensitivity and specificity were determined considering true positives all Kaposi's sarcoma (KS) sera and using three different cut-offs to consider as true positives the serum samples from individuals without KS (positive score with at

least 70%, 80%, or 90% of the nine assay). White bars, 70% cut off; grey bars, 80% cut off; black bars, 90% cut off. The sensitivity and specificity of the combination of Rlyt and LLANA IFA (Rlyt-LLANA) as calculated with the exclusion criteria is also shown (see text for details).

model and the odds ratios (OR) were determined. Since each OR computes both the sensitivity and specificity characteristics of a given assay, this analysis provides estimates of the assay validity. Each OR, in addition, represents the ratio of the probability for any individual to be HHV-8 infected if positive in a given serological assay and the probability to be infected if negative in the assay. Thus, the OR values provide also estimates of the predictive value of the assays, a parameter that cannot be determined directly due to the lack of knowledge of the real prevalence of HHV-8 infection in the population [Choi, 1997].

All the nine assays had OR values significantly greater than one ($P < 0.05$) (Table II). In this setting, the Rome lytic antigen IFA appeared to be the most valid assay, with OR ranging from 135 to 167 under the three different assumptions. This means that individuals who have a Rlyt positive test are 135 to 167 times more likely to be infected than individuals negative with this assay. High OR values (above 50) were also found for the Munich peptide mix ELISA M2, the Erlangen orfK8.1 ELISA, and the Liverpool latent nuclear antigen IFA. Table II assigns to each assay a validity rank according to their OR. The validity of the

TABLE II. Results of the Univariate Logistic Regression Analysis Showing OR (CI in Parenthesis) and Validity Rank*

Assay	> 70%	> 80%	> 90%	Compressive validity rank
Rlyt	167 (40–697)	143 (34–599)	135 (35–563)	1
M2	100 (45–222)	88 (39–198)	79 (35–176)	2
EK8.1	81 (38–172)	59 (28–123)	53 (25–108)	3
LLANA	68 (29–159)	57 (25–131)	53 (23–122)	4
L65	20 (10–43)	18 (28–123)	17 (8–35)	5
EK12	15 (8–26)	16 (8–29)	14 (7–26)	6
L73	9 (5–16)	8 (5–14)	8 (5–14)	7
E65	7 (4–12)	8 (4–13)	7 (4–12)	8
Rlat	8 (5–15)	7 (4–12)	6 (3–12)	9

*The table shows the results of a univariate logistic regression model performed to evaluate the validity of the various assays. The model permits calculating the odds ratios (OR) that represents the risk of any individual with a positive test to be infected as compared to the risk of individuals with a negative test. The model accounted for correlation among repeated measurements in the same individuals. The calculations to determine the OR were repeated using the three different proportions of sera considered to be true positives [i.e., all Kaposi's sarcoma (KS) sera plus non-KS sera scoring positive with at least 70%, 80%, or 90% of the nine assays used to screen all the five serum panels].

Stuttgart BCBL-1 IFA, the Biotrin IFA and the Basel orf65 ELISA were evaluated on the basis of the data obtained by screening only the two panels Munich 1 and Munich 2. These assays yielded OR values ranging between 13.3 and 24.1 ($P < 0.05$) (data not shown).

The OR values indicated that some of the assays, particularly the Rome lytic antigen IFA, the Munich peptide mix ELISA M2, the Erlangen orfK8.1 ELISA, and the Liverpool latent nuclear antigen IFA had good sensitivity and specificity characteristics and high predictive value. This suggested a combination of these assays to obtain pairs of assays with improved validity. For this purpose, two different combination criteria were used. One criterion considered as positive the sera that scored positive with either one of the assays of the combination (inclusion criterion); the other considered positive only the sera that scored positive with both assays (exclusion criterion).

When the inclusion criterion was used, the balance of sensitivity and specificity of the combinations of assays did not improve significantly as compared to the most sensitive assays (data not shown). In contrast, the use of the exclusion criterion identified a combination of assays (the Liverpool latent nuclear antigen and the Rome lytic antigen IFAs) characterised by a increased balance of sensitivity (89.1%) and specificity (94.9%) values as compared to the four best single assays (Fig. 2A, B).

DISCUSSION

Sensitive and reliable serological assays to detect HHV-8 infection are required to assess the global dimensions of HHV-8 infection and virus pathogenicity, to compare findings from different geographical areas, to facilitate the diagnostic process, and to implement the control of HHV-8-associated diseases, particularly those associated to HIV infection and organ transplantation.

To date, only one study by Rabkin et al. [1998] has been published showing a rather low concordance between first generation assays. Our study, which compared the performance of available or novel serological assays and combinations of assays on a large number of sera from KS patients, HIV positive and negative individuals, and blood donors, shows significant improvements. In this study, which was conducted in seven European laboratories, 18 different assays, both IFA and ELISA, were evaluated. Two IFAs, the Rome lytic antigen and the Liverpool latent nuclear antigen IFA (detecting antibodies against lytic or latent HHV-8 antigens, respectively), and two ELISAs, the Munich peptide mix ELISA M2 and the Erlangen orfK8.1 ELISA (detecting antibodies against HHV-8 peptides with a high antigenic index or against the HHV-8 ORF K8.1 protein, respectively), showed a good balance of sensitivity and specificity, high OR values indicative of a high predictive value, and a good agreement when compared to each other. With sera from blood donors, the Munich peptide mix ELISA M2

tended to provide lower seroprevalence rates as compared with the other assays showing high sensitivity values with KS sera. The Rome lytic antigen IFA identified as positive a lower proportion of Ugandan sera (56% vs. 77%) or non-Mediterranean HIV negative donors [13 out of 88 (6.9%)] as compared to a lytic IFA described previously [Lennette et al., 1996] (data not shown). This probably reflects the different experimental procedures and read-out criteria used in the two laboratories. In general, the concordance for blood donor sera was lower than for KS sera but significantly higher as compared to that reported by Rabkin et al. [1998].

The sensitivity of most assays used in our comparative study and the inter-assay concordance measured on blood donors appeared to be higher than that reported for first generation assays. However, some disappointment derives from the wide variation observed in the HHV-8 seroprevalence among KS-free HIV positive individuals, though the κ statistics was significant in 58.3% of the couples as compared to the 38.1% of the study by Rabkin and co-workers cited above (data not shown). Furthermore, the reliability of the assays was not satisfactory when used on Ugandan samples. The reason for this finding is unknown and may be due to cross-reactions with antibodies directed against other infectious agents.

Reliable diagnostic tools for HHV-8 infection are required for the clinical management of HIV infected individuals as the risk of KS development is maximal when HHV-8 seroconversion occurs in the setting of an established HIV infection [Renwick et al., 1998; Rezza et al., 1999]. The diagnosis of HHV-8 infection, however, is probably even more important for organ transplantation due to the fact that the risk of developing HHV-8 associated disease is related directly to the proper management of the transplantation procedures including the identification of the organ donor [Hudnall et al., 1998; Regamey et al., 1998b; Dotti et al., 1999; Farge et al., 1999; Parravicini et al., 1999]. Serological assays with high sensitivity and specificity, such as the Munich peptide mix ELISA M2, the Liverpool latent nuclear antigen IFA, the Erlangen orfK8.1 ELISA, or the combination of the Rome lytic antigen and the Liverpool latent nuclear antigen IFAs, are required for screening of organ recipients. Since the risk of KS development is exceedingly high for HHV-8 seropositive organ recipients receiving allografts from HHV-8 seropositive organ donors [Parravicini et al., 1999], HHV-8 positive recipients should be matched with a negative donor. Tests with a very high sensitivity can be used to safely exclude HHV-8 positive donors whereas highly specific assays will yield fewer false positives organ donors. Thus, the final choice should also consider the availability of organs for donation and the future risks related to the development of KS in relation to the general conditions of the organ recipient. ELISAs are particularly useful tests for routine work because the correct interpretation of IF tests relies largely on the experience of the

observers. In particular, it is important to point out that we have observed a high degree of variability in the performance of different lytic IFAs, probably due to differences in technical protocols and read out in different laboratories (data not shown).

Before drawing any firm conclusion, some limitations and possible biases of the study should be mentioned. First, out of 18 assays only 9 were used on all serum panels. However, the data generated by using the other assays for the screening of subsets of the 5 panels were not in conflict with the results presented in this study (data not shown). Second, since a gold standard was not available, simulations were used to describe the characteristics of the assays. In particular, to calculate the sensitivity and specificity of the assays, we assumed that the true positive sera included those from KS patients (who are all infected by HHV-8) and the samples positive with at least 70%, 80%, or 90% of the assays. A regression model was applied to the data to evaluate the validity and predictive value of the assays [Choi, 1997]. Finally, none of the assays described in this paper was standardised; therefore, their use in clinical practice has to be considered with caution, since in the lack of standardised procedures the inter-observer agreement on the same test may not be good.

In conclusion, although none of the serological tests for HHV-8 can yet be considered a gold standard, we have defined tests with a high sensitivity, specificity, and predictive value, which can be useful diagnostic tools for further research and control activities.

ACKNOWLEDGMENTS

We thank A. Lippa and F. M. Regini for the editorial work. This work was supported by grants from the Istituto Superiore di Sanità "III National Research Programme on AIDS" to B.E. and G.R., "III National Research Programme on AIDS"-Concerted Action "Epidemiology" to M.A., and the "Uganda AIDS Project" (all from the Italian Ministry of Health), by a grant from the Associazione Italiana per la Ricerca sul Cancro (AIRC) to B.E., from the Friedrich-Baur-Stiftung (RK 8233049) to O.S., NHS Biomedical Research Frame RDO/22/09' to T.F.S., Swiss National Science Funds Nr. 31-064233.00 to P.E., Deutsche Forschungsgemeinschaft SFB 466: "Lymphoproliferation and Viral Immunodeficiency" to F.N., and the European Concerted Action "Pathogenesis of AIDS-KS."

REFERENCES

- Andreoni M, El Sawaf G, Rezza G, Ensoli B, Nicastrì E, Ventura L, Ercoli L, Sarmati L, Rocchi G. 1999. High seroprevalence of antibodies to human herpesvirus-8 in Egyptian children: evidence of nonsexual transmission. *J Natl Cancer Inst* 91:465-469.
- Blauvelt A, Sei S, Cook PM, Schulz TF, Jeang KT. 1997. Human herpesvirus 8 infection occurs following adolescence in the United States. *J Infect Dis* 176:771-774.
- Boshoff C, Schulz TF, Kennedy MM, Graham AK, Fisher C, Thomas A, McGee JO, Weiss RA, O'Leary JJ. 1995. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat Med* 1:1274-1278.
- Bourbouliia D, Whithy D, Boshoff C, Newton R, Beral V, Carrara H, Lane A, Sitas F. 1998. Serological evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus infection. *Jama* 280:31-32.
- Calabrò ML, Sheldon J, Favero A, Simpson GR, Fiore JR, Gomes E, Angarano G, Chieco Bianchi L, Schulz TF. 1998. Seroprevalence of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 in several regions of Italy. *J Hum Virol* 1:207-213.
- Calabrò ML, Fiore JR, Favero A, Lepera A, Saracino A, Angarano G, Schulz TF, Chieco-Bianchi L. 1999. Detection of human herpesvirus 8 in cervicovaginal secretions and seroprevalence in human immunodeficiency virus type 1-seropositive and -seronegative women. *J Infect Dis* 179:1534-1537.
- Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* 332:1186-1191.
- Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266:1865-1869.
- Choi BC. 1997. Causal modeling to estimate sensitivity and specificity of a test when prevalence changes. *Epidemiology* 8:80-86.
- Decker LL, Shankar P, Khan G, Freeman RB, Dezube BJ, Lieberman J, Thorley Lawson DA. 1996. The Kaposi sarcoma-associated herpesvirus (KSHV) is present as an intact latent genome in KS tissue but replicates in the peripheral blood mononuclear cells of KS patients. *J Exp Med* 184:283-288.
- Dotti G, Fiocchi R, Motta T, Facchinetti B, Chiodini B, Borleri GM, Gavazzoni G, Barbui T, Rambaldi A. 1999. Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus-8. *Leukemia* 13:664-670.
- Dupon M, Masquelier B, Cazorla C, Chene G, Dumon B, Ragnaud JM, de Barbeyrac B, Bébear C, Lacut JY, Fleury HJ. 1997. Acquired immunodeficiency syndrome-associated Kaposi's sarcoma and human herpesvirus 8 DNA detection in serial peripheral blood mononuclear cell samples. *Res Virol* 148:417-425.
- Ensoli B, Stürzl M. 1998. Kaposi's sarcoma: a result of the interplay among inflammatory cytokines, angiogenic factors and viral agents. *Cytokine Growth Factor Rev* 9:63-83.
- Farge D, Lebbé C, Marjanovic Z, Tuppin P, Mouquet C, Peraldi MN, Lang P, Hiesse C, Antoine C, Legendre C, Bedrossian J, Gagnadoux MF, Loirat C, Pellet C, Sheldon J, Golmard JL, Agbalika F, Schulz TF. 1999. Human herpes virus-8 and other risk factors for Kaposi's sarcoma in kidney transplant recipients: Groupe Cooperatif de Transplantation d'Ile de France (GCIF). *Transplantation* 67:1236-1242.
- Fleiss JL. 1981. The measurement of interrater agreement. In: *Statistical methods for rates and proportions*, 2nd edition. New York: John Wiley & Sons, Inc. p 212-236.
- Gaidano G, Pastore C, Gloghini A, Cusini M, Nomdedeu J, Volpe G, Capello D, Vaccher E, Bordes R, Tirelli U, Saglio G, Carbone A. 1996. Distribution of human herpesvirus-8 sequences throughout the spectrum of AIDS-related neoplasia. *Aids* 10:941-949.
- Gao SJ, Kingsley L, Hoover DR, Spira TJ, Rinaldo CR, Saah A, Phair J, Detels R, Parry P, Chang Y, Moore PS. 1996a. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N Engl J Med* 335:233-241.
- Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo CR, Saah A, Phair J, Detels R, Chang Y, Moore PS. 1996b. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nat Med* 2:925-928.
- Grandadam M, Dupin N, Calvez V, Gorin I, Blum L, Kernbaum S, Sicard D, Buisson Y, Agut H, Escande JP, Huraux JM. 1997. Exacerbations of clinical symptoms in human immunodeficiency virus type 1-infected patients with multicentric Castlemann's disease are associated with a high increase in Kaposi's sarcoma herpesvirus DNA load in peripheral blood mononuclear cells. *J Infect Dis* 175:1198-1201.
- Harrington WJ Jr, Bagasra O, Sosa CE, Bobroski LE, Baum M, Wen XL, Cabral L, Byrne GE, Pomerantz RJ, Wood C. 1996. Human herpesvirus type 8 DNA sequences in cell-free plasma and mononuclear cells of Kaposi's sarcoma patients. *J Infect Dis* 174:1101-1105.
- Hudnall SD, Rady PL, Tying SK, Fish JC. 1998. Serological and molecular evidence of human herpesvirus 8 activation in renal transplant recipients. *J Infect Dis* 178:1791-1794.
- Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. 1996. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-

- associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat Med* 2:918–924.
- Knowles DM, Cesarman E. 1997. The Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in Kaposi's sarcoma, malignant lymphoma, and other diseases. *Ann Oncol* 8:123–129.
- Lang D, Hinderer W, Rothe M, Sonneborn HH, Neipel F, Raab M, Rabenau H, Masquelier B, Fleury H. 1999. Comparison of the immunoglobulin-G-specific seroreactivity of different recombinant antigens of the human herpesvirus 8. *Virology* 260:47–54.
- Lennette ET, Blackbourn DJ, Levy JA. 1996. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. *Lancet* 348:858–861.
- Maclure M, Willet WC. 1987. Misinterpretation and misuse of the kappa statistic. *Am J Epidemiol* 126:161–169.
- Monini P, Colombini S, Stürzl M, Goletti D, Cafaro A, Sgadari C, Buttò S, Franco M, Leone P, Fais S, Leone P, Melucci Vigo G, Chiozzini C, Carlini F, Ascherl G, Cornali E, Zietz C, Ramazzotti E, Ensoli F, Andreoni M, Pezzotti P, Rezza G, Yarchoan R, Gallo RC, Ensoli B. 1999. Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. *Blood* 93:4044–4058.
- Moore PS, Kingsley LA, Holmberg SD, Spira T, Gupta P, Hoover DR, Parry JP, Conley LJ, Jaffe HW, Chang Y. 1996. Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. *Aids* 10:175–180.
- Olsen SJ, Chang Y, Moore PS, Biggar RJ, Melbye M. 1998. Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985. *Aids* 12:1921–1925.
- Parravicini C, Poli F, Bestetti G, Lauri E, Sirchia G, Rossi E, Berti E, Piazza M, Sciamogna C, Moroni M, Galli M, Corbellino M. 1999. Risk of Kaposi's sarcoma development in renal transplant patients from Northern Italy. The second International Workshop on KSHV/HHV-8 and related agents. Oxford, UK.
- Raab MS, Albrecht JC, Birkmann A, Yaguboglu S, Lang D, Fleckenstein B, Neipel F. 1998. The immunogenic glycoprotein gp35–37 of human herpesvirus 8 is encoded by open reading frame K8.1. *J Virol* 72:6725–6731.
- Rabkin CS, Schulz TF, Whitby D, Lennette ET, Magpantay LI, Chatlynne L, Biggar RJ. 1998. Interassay correlation of human herpesvirus 8 serological tests. HHV-8 Interlaboratory Collaborative Group. *J Infect Dis* 178:304–309.
- Rainbow L, Platt GM, Simpson GR, Sarid R, Gao SJ, Stoiber H, Herrington CS, Moore PS, Schulz TF. 1997. The 222- to 234-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. *J Virol* 71:5915–5921.
- Regamey N, Cathomas G, Schwager M, Wernli M, Harr T, Erb P. 1998a. High human herpesvirus 8 seroprevalence in the homosexual population in Switzerland. *J Clin Microbiol* 36:1784–1786.
- Regamey N, Tamm M, Wernli M, Witschi A, Thiel G, Cathomas G, Erb P. 1998b. Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. *N Engl J Med* 339:1358–1363.
- Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D. 1996. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. *Nat Med* 2:342–346.
- Renwick N, Halaby T, Weverling GJ, Dukers NH, Simpson GR, Coutinho RA, Lange JM, Schulz TF, Goudsmit J. 1998. Seroconversion for human herpesvirus 8 during HIV infection is highly predictive of Kaposi's sarcoma. *Aids* 12:2481–2488.
- Rezza G, Andreoni M, Dorrucchi M, Pezzotti P, Monini P, Zerboni R, Salassa B, Colangeli V, Sarmati L, Nicastrì E, Barbanera M, Pristeri R, Aiuti F, Ortona L, Ensoli B. 1999. Human herpesvirus 8 seropositivity and risk of Kaposi's sarcoma and other acquired immunodeficiency syndrome-related diseases. *J Natl Cancer Inst* 91:1468–1474.
- Schulz TF. 1999. Epidemiology of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8. *Adv Cancer Res* 76:121–160.
- Simpson GR, Schulz TF, Whitby D, Cook PM, Boshoff C, Rainbow L, Howard MR, Gao SJ, Bohenzky RA, Simmonds P, Lee C, de Ruiter A, Hatzakis A, Tedder RS, Weller IV, Weiss RA, Moore PS. 1996. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* 348:1133–1138.
- Sitas F, Newton R, Boshoff C. 1999. Increasing probability of mother-to-child transmission of HHV-8 with increasing maternal antibody titer for HHV-8. *N Engl J Med* 340:1923.
- Smith NA, Sabin CA, Gopal R, Bourbouli D, Labbet W, Boshoff C, Barlow D, Band B, Peters BS, de Ruiter A, Brown DW, Weiss RA, Best JM, Whitby D. 1999. Serological evidence of human herpesvirus 8 transmission by homosexual but not heterosexual sex. *J Infect Dis* 180:600–606.
- Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals HD, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L, Sigaux F. 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castlemann's disease. *Blood* 86:1276–1280.
- Stürzl M, Blasig C, Schreier A, Neipel F, Hohenadl C, Cornali E, Ascherl G, Esser S, Brockmeyer NH, Ekman M, Kaaya EE, Tschachler E, Biberfeld P. 1997. Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma. *Int J Cancer* 72:68–71.
- Whitby D, Howard MR, Tenant-Flowers M, Brink NS, Copas A, Boshoff C, Hatzioannou T, Suggett FEA, Aldam DM, Denton AS, Miller RF, Weller IVD, Weiss RA, Tedder RS, Schulz TF. 1995. Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* 346:799–802.