Real-Time Epstein-Barr virus PCR for the diagnosis of primary EBV infection and EBV reactivation

Article *in* Molecular Diagnosis · December 2005

DOI: 10.2165/00066982-200509040-00005 · Source: PubMed

6 authors, including:

Hubert G M Niesters
University of Groningen
404 PUBLICATIONS **14,530** CITATIONS
SEE PROFILE

Steven F T Thijsen
Diakonessenhuis Utrecht
145 PUBLICATIONS **3,347** CITATIONS
SEE PROFILE

ORIGINAL RESEARCH ARTICLE

Mol Diagn 2005; 9 (4): 195-200
1084-8592/05/0004-0195/$34.95/0

Real-Time Epstein-Barr Virus PCR for the Diagnosis of Primary EBV Infections and EBV Reactivation

Rianne Luderer,[1] *Marieke Kok*,[2] *Hubert G.M. Niesters*,[3] *Rob Schuurman*,[4] *Okke de Weerdt*[5] and *Steven F.T. Thijsen*[2]

1 Unit Molecular Diagnostics, Diakonessenhuis Utrecht, Utrecht, The Netherlands
2 Department of Medical Microbiology, Diakonessenhuis Utrecht, Utrecht, The Netherlands
3 Department of Virology, University Medical Center Rotterdam, Rotterdam, The Netherlands
4 Department of Virology, Eijkman-Winkler Institute for Microbiology, Infectious Diseases and Inflammation, University Medical Center Utrecht, Utrecht, The Netherlands
5 Department of Hematology, St Antonius Hospital, Nieuwegein, The Netherlands

Abstract

Background: The serological diagnosis of primary Epstein-Barr virus (EBV) infections is often difficult, whereas the relevance of elevated immunoglobulin G (IgG) antibodies against early antigen (EA) for the diagnosis of EBV reactivation has increasingly become a matter of dispute. Recently, EBV PCR has been added as a diagnostic tool. Positive EBV PCR has been demonstrated in the serum of patients with primary EBV infections and EBV reactivation.

Objectives: To compare classical serological diagnosis of primary EBV infection and EBV reactivation with real-time EBV PCR.

Study design: Sera from 45 patients were selected with detectable immunoglobulin M (IgM) antibodies against EBV viral capsid antigen (VCA), and 62 sera were selected with a reactivation profile. A real-time EBV PCR was performed with DNA extracted from these sera.

Results: Based on serological data, the diagnosis of primary EBV infection was established for 24 of the 45 IgM VCA-positive patients. By performing PCR, seven extra cases of primary infection were diagnosed for which no heterophilic antibodies could be detected. In five cases of primary infection, no EBV DNA could be detected by PCR. Only in two of the 62 sera with a reactivation seroprofile could EBV DNA be detected.

Conclusions: Based on these data, we suggest that for the diagnosis of primary infections, EBV PCR could lead to an increase of >16% in the number of positive diagnoses by confirming a positive IgM VCA in the absence of heterophilic antibodies. Furthermore, EBV PCR is positive in only 3% of sera with elevated antibodies against EA, raising doubt as to the utility of EA titers for diagnosing EBV reactivation.

Epstein-Barr virus (EBV) infections occur worldwide and affect >90% of the population at adulthood. Despite this high prevalence, diagnosis of a primary infection or reactivation can still pose a problem. The diagnosis of primary EBV infection relies on the detection of heterophilic antibodies and/or immunoglobulin M (IgM) antibodies against viral capsid antigen (VCA) in the absence of antibodies against EBV nuclear antigen (EBNA).[1] The serological diagnosis of EBV reactivation is less clear, but is often linked to a seroprofile with elevated titers against early antigen (EA) of EBV in conjunction with detectable immunoglobulin G (IgG) antibodies against EBNA and undetectable IgM antibodies against VCA.[2-6]

Several problems may arise while interpreting serological results for primary EBV infections. Heterophilic antibodies are not

Table I. Patient demographics, Epstein-Barr virus (EBV) serology and EBV PCR in the immunoglobulin M (IgM)-positive group

Parameter	IgM-positive group		
	total	EBV PCR	
		positive	negative
Sex			
male	17	9	8
female	28	17	11
Age (years)			
median	23	15	31
range	2–90	2–49	4–90
Serology			
IgG EBNA (positive/total tested)	13/45	0/26	13/19
Paul-Bunnell (positive/total tested)	13/45	10/26	3/19
Monospot (positive/total tested)	25/45	19/26	6/19

EBNA = EBV nuclear antigen; **IgG** = immunoglobulin G.

produced reliably after a primary infection in young children, but in some cases they can be detected months after infection.[7] The Paul-Bunnell test is considered a 'gold standard' test for the detection of heterophilic antibodies and is often used to confirm the results of less labor-intensive quick tests such as the Monospot (Meridian Bioscience, Inc., Cincinnati, OH, USA), since these tests can produce false-positive results.[8,9] Positive IgM VCA results can occur as a result of cross-reactivity in patients with autoantibodies[10] or because of cross-reaction with other herpesviruses such as cytomegalovirus.[11-13] In addition, IgG antibodies against EBNA can be detected in the sera of patients with a primary EBV infection.[7]

The significance of elevated EA titers in patients suspected of EBV reactivation dates back to literature of the 1970s and 1980s.[2-6] In these studies, however, virus replication was not documented. Later studies demonstrated that elevated EA titers are probably non-specific.[14-19] Soto and Straus[19] concluded that the role of EBV reactivation, as diagnosed by elevated EA titers in patients complaining of fatigue and other non-specific signs, is fading rapidly.

Recently, several studies have demonstrated that EBV DNA can be detected in the serum of most patients with a primary EBV infection.[7,20-25] In addition, the clinical value of an EBV DNA quantification test in serum for the diagnosis of EBV reactivation in immunocompromised patients has been firmly established.[26,27] Taking into consideration the often difficult serological diagnosis of EBV infections, we investigated the value of EBV DNA detection using real-time amplification in support of the serological diagnosis of primary EBV infection and EBV reactivation.

Methods

Patients and Materials

Two groups of sera, tested routinely for EBV between 2001 and 2003 and stored at −20°C, were selected: 45 sera with detectable IgM antibodies against EBV VCA; and 62 sera with a reactivation profile with detectable IgG antibodies against EA in combination with IgG antibodies against EBNA but no IgM antibodies against VCA. The exact time interval between the onset of symptoms and sampling could not be documented because of the retrospective design of the study. If more than one serum from the same patient was available, the first one was selected.

Three different ELISAs were used to detect IgM antibodies against EBV VCA, IgG antibodies against EBNA, and IgG antibodies against EA: from January 2001 to April 2001, Gull Diagnostics (DenBosch, The Netherlands); from April 2001 to November 2001, Biotest Seralc NV (Soest, The Netherlands); and from November 2001 to December 2002, Meridian Bioscience, Inc. (Cincinnati, OH, USA). Heterophilic antibodies were determined using the Monospot test and the Paul-Bunnell test. The Paul-Bunnell test was performed using sheep erythrocytes after an absorption step against guinea pig kidney cells, and a cut-off value of 1 : 64 was applied.[28,29]

PCR and DNA Isolation

DNA was isolated from 200µL of sample material using the QIAamp DNA Mini Kit (QIAGEN GmbH, Düsseldorf, Germany) according to the instructions of the manufacturer and eluted in 100µL. A quantitative real-time EBV PCR, targeting the nonglycosylated membrane antigen *BNRF p143* gene of EBV, was performed in duplicate on each sample, as described previously,[30] using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Quantification was performed using standard curves derived from an electron microscope-counted stock of EBV (EBV B95-8, Advanced Biotechnologies Inc, Colombia, MD, USA) ranging from 50 to 5×10^7 copies per mL. The assay results were linear within this range and 50 copies per mL was detected in 71% of PCRs. A mean standard curve was calculated based on the average cycle threshold (Ct) values of 12 standard curves.

As an internal control, all samples were spiked with phocine herpesvirus type 1 (PhHV-1).[30] The average Ct value obtained for

PhHV was 27.27 ± 0.425. Amplification of samples was considered to be inhibited when the Ct value for PhHV-1 differed by more than three standard deviations from the longstanding average. EBV PCR results were regarded as positive when both duplicate reactions generated a detectable Ct value. The experiment was repeated if only one of the duplicate reactions was positive. Each extraction round and each PCR run included positive and negative control samples. None of the negative control reactions produced signals above the threshold value for the fluorescence signal, indicating that no false-positive results were encountered.

Results

Primary Infection

Real-time EBV PCR was performed on 45 IgM VCA-positive sera. Patient demographics and EBV serology results are presented in table I. EBV DNA was detected in 26 of these 45 sera (58%), with a mean viral load of 1099 copies per mL, a median of 427 copies per mL and a range of 29–9896 copies per mL. The mean age in this group was significantly lower as compared with the PCR-negative group (table I).

Of the 45 IgM VCA-positive sera, 24 showed a seroprofile matching a primary infection: detection of heterophilic antibodies with the Monospot test in combination with the presence of IgM VCA antibodies and the absence of IgG EBNA antibodies. In total, 25 sera were positive in the Monospot test (table I), suggesting one false-positive result. Twelve of the 25 sera with a positive Monospot result were negative in the Paul-Bunnell test. Seven of these 12 samples had titers in the Paul-Bunnell test of 1 : 32, just below the cut-off. All of these seven sera had a positive EBV PCR result. Two of the remaining five sera with a non-reactive Paul-Bunnell test and a positive Monospot test also had a positive EBV PCR result, indicating greater sensitivity of the Monospot test as compared with the Paul-Bunnell test. The other three samples in this group had a negative EBV PCR result. All samples with a positive PCR result had undetectable antibodies against EBNA (table I).

In eight IgM VCA-positive and IgG EBNA-negative sera, no heterophilic antibodies (measured by both the Monospot test and the Paul-Bunnell test) could be detected, whereas the EBV PCR was positive in seven of these sera (table II). The mean age in this PCR-positive heterophilic antibody negative group was significantly lower as compared with the heterophilic antibody-positive group: 9 versus 20 years ($p = 0.028$).

Reactivation

Reactivation sera (detectable IgG antibodies against EA in combination with IgG antibodies against EBNA but no IgM antibodies against VCA) were divided into two groups: 27 sera with a strong positive reaction against EA (>2.5 times the cut-off value); and 35 sera with a weak positive reaction against EA (<2.5 times the cut-off value) [table III]. IgG reactivity against VCA was comparable in both groups. In the first group, 26 of 27 sera (96%) were PCR negative, and one serum tested PCR positive with a viral load of 112 copies per mL. This sample was derived from a patient with a mantle cell lymphoma. In the second group, 34 of 35 (97%) were PCR negative and one serum was PCR positive with a viral load of 108 864 copies per mL. This patient had an EBV lymphoproliferative disease (EBV-LPD), which emerged after treatment for non-Hodgkin's lymphoma (NHL).

Table II. Discrepancies: immunoglobulin M (IgM) viral capsid antigen (VCA) and Epstein-Barr virus (EBV) PCR vs heterophilic antibodies

Serum	Age (years)	Sex	IgG EBNA	IgM VCA	Paul Bunnell test	Monospot test	EBV PCR (copies per mL)
1	3	Male	−	+	−	−	29
2	15	Female	−	+	−	−	368
3	9	Female	−	+	−	−	388
4	5	Male	−	+	−	−	447
5	22	Female	−	+	−	−	814
6	8	Female	−	+	−	−	1683
7	2	Female	−	+	−	−	9896
8	14	Male	−	+	−	−	Undetectable

EBNA = EBV nuclear antigen; **IgG** = immunoglobulin G; **+** indicates positive; **−** indicates negative.

Table III. Patient demographics, Epstein-Barr virus (EBV) serology and EBV PCR in the reactivation group

Parameter	Reactivation group		
	total	EBV PCR	
		positive	negative
Sex			
male	19	1	18
female	43	1	42
Age (years)			
median	41	54	41
range	6–64	47–60	6–64
Serology			
IgG early antigen strong positive	27	1	26
IgG early antigen weak positive	35	1	34

IgG = immunoglobulin G.

Discussion

We detected EBV DNA in 26 of 45 (58%) IgM VCA-positive sera. A seroprofile with positive IgM VCA antibodies, in combination with a positive heterophil antibody quick test such as the Monospot test and negative IgG antibodies for EBNA, makes a primary EBV infection likely. Using this definition, we were able to detect 24 primary infections in a group of 45 IgM VCA-positive patient sera (53%). If a positive EBV PCR in serum with positive IgM antibodies against VCA and negative IgG antibodies against EBNA is also considered as strong evidence of a primary infection, then a primary infection was diagnosed in 31 of 45 sera (69%). This translates to a 16% rise in the number of diagnoses of primary infections as compared with our current serology-based diagnostic approach. This increase was a result of the detection of EBV DNA in patients (mainly children) who failed to produce detectable levels of heterophilic antibodies (table II). EBV PCR on serum was positive in 26 of these 31 cases (84%) with a primary infection. Conversely, in 5 of 31 sera diagnosed as primary EBV infections, PCR was negative (16%). EBV PCR was always negative in patients with a positive IgM VCA and a positive IgG EBNA, indicating that EBV PCR is not useful in those cases.

In other studies, comparable numbers of EBV-positive sera have been reported (table IV).[22-25,30-32] Berger et al.[22] analyzed sera from children with a primary infection and found a positive EBV PCR in all 49 sera taken within 14 days after the onset of disease. In most patients, a rapid decline of EBV viral load was observed within 2–25 days. Kimura et al.[32] demonstrated that children with a primary infection had a positive EBV PCR in 19 of 20 cases within 10 days after the first day of illness, with an average copy number of 251 geq/mL. Between days 11 and 20 after the onset of disease, six of eight patients still had a positive PCR; 20 days after onset, one of five sera still tested positive in the PCR. Taking all these studies together, it demonstrates that in the majority of patients with a primary EBV infection, EBV DNA can be detected, although the window of PCR positivity is relative short, limiting the applicability of EBV PCR for diagnosing an acute infection.

A positive IgM VCA in combination with undetectable antibodies against EBNA and a positive EBV PCR can be considered as proof of a primary infection. Using this definition, we were able to detect the presence of a primary infection in 26 cases. Paul-Bunnell tests were negative in 16 of these 26 cases (62%) and the Monospot test was negative in 7 of these 26 cases (27%). This, combined with the low false-positive rate of the Monospot (one possible case of 45), led us to conclude that the Monospot should not be confirmed with the Paul-Bunnell to avoid false-negative results.

Subsequently, we analyzed 62 sera with a reactivation seroprofile and could only detect EBV DNA in two patients: one patient with an EBV-LPD after treatment of a primary NHL; and the other with a mantle cell lymphoma. EBV DNA in the serum of patients with NHL has already been described by others.[33,34] Chan et al.[23] suggested that any EBV reactivation is likely to be accompanied by a viremia. Elaborating on this hypothesis, one could conclude that testing for IgG antibodies against EA leads to a false positivity rate of >95%. Testing for EA would therefore pose the risk that patients would be sent home with a diagnosis of EBV reactivation rather than having their underlying illness investigated further, leading to diagnostic delay.

Berger et al.[22] were able to demonstrate a positive EBV PCR on serum in 9 of 51 samples (18%) with a seroprofile matching a past infection: detectable IgG antibodies against VCA and EBNA and undetectable IgM antibodies against VCA. Copy numbers varied between 100 and 2980 per mL. This finding is in contrast to our own observation and those of others[24,30,35] who could not find a positive EBV PCR in the sera of patients with evidence of a past infection. The PCR applied by Berger et al.[22] targets the *Bam* HI W region of the EBV genome. Blast analysis revealed that, in contrast to the PCR applied in this study,[30] both the primers and probe of the PCR applied by Berger et al.[22] were 100% identical to human DNA (National Center for Biotechnology Information [NCBI] accession number BX248579). This identity might be a result of integration of EBV DNA into the human genome as has been described in patients with chronic active EBV infections and EBV-associated diseases.[36,37] Consequently, traces

Table IV. Epstein-Barr virus (EBV) PCR of serum samples

Study	Technique	Number of patients	% of positives	Viral load (copies per mL)	
				mean	range
Gan et al.,[25]	PCR/blot	41	27	ND	ND
Laroche et al.,[31]	PCR/gel	19	68	ND	ND
Chan et al.,[23]	PCR/gel	46	80	ND	ND
Niesters et al.,[30]	Taqman	22	73	6400	<100–45 000
Kimura et al.,[32]	Taqman	20	95	251	ND
Berger et al.,[22]	Taqman	49	100	3847	16–71 104
Brengel-Pesce et al.,[24]	Lightcycler	15	80	288	ND
Total		**212**			
Average			74		

ND = no data.

of human DNA with integrated EBV DNA might result in false-positive reactions when the EBV PCR described by Berger et al.[22] is applied. Furthermore, the PCR applied by Berger et al.[22] is part of the large internal repeat sequence of EBV.[38] A PCR directed against this repeat will be more sensitive than a PCR directed to a single copy part of the EBV genome. This could be an alternative explanation as to why Berger et al.[22] found a higher percentage of positive EBV PCRs when analyzing serum derived from patients with a latent EBV infection. Since the copy number of the long-terminal repeat is, however, not constant, this target is unsuitable for quantitative PCR.[39]

Interestingly, we were able to observe clear EBV amplification signals in one of the duplicate EBV PCRs in 4 of 107 sera (4%) at low copy numbers, whereas repeated analyses of these samples showed that the presence of EBV DNA could not be substantiated. As suggested by Kimura et al.,[32] these results could be explained by DNA from latently infected lysed B cells present in the serum.

In conclusion, we propose that in the case of a suspected primary EBV infection, both EBV-specific serology and a heterophil antibody test, using a quick test such as the Monospot, should be performed. In the case of a negative Monospot, a positive IgM VCA and a negative IgG EBNA, one cannot exclude the possibility of a false-positive IgM VCA. An EBV PCR should be considered in these cases, which may lead to a 16% increase in definitive diagnoses of primary EBV infection. In sera with an EBV reactivation seroprofile, EBV DNA could only be detected in a small minority of samples, which, in our opinion, is further evidence against the diagnostic value of EA antibodies in patients with suspected EBV reactivation.

Acknowledgments

No sources of funding were used to assist in conducting this study. The authors have no conflicts of interest that are directly relevant to the content of the study.

References

1. Grotto I, Mimouni D, Huerta M, et al. Clinical and laboratory presentation of EBV positive infectious mononucleosis in young adults. Epidemiol Infect 2003; 131 (1): 683-9
2. Horwitz CA, Henle W, Henle G, et al. Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. Am J Med 1975; 58 (3): 330-8
3. Tobi M, Morag A, Ravid Z, et al. Prolonged atypical illness associated with serological evidence of persistent Epstein-Barr virus infection. Lancet 1982; I (8263): 61-4
4. DuBois RE, Seeley JK, Brus I, et al. Chronic mononucleosis syndrome. South Med J 1984; 77 (11): 1376-82
5. Jones JF, Ray CG, Minnich LL, et al. Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. Ann Intern Med 1985; 102 (1): 1-7
6. Straus SE, Tosato G, Armstrong G, et al. Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. Ann Intern Med 1985; 102 (1): 7-16
7. Rea TD, Ashley RL, Russo JE, et al. A systematic study of Epstein-Barr virus serologic assays following acute infection. Am J Clin Pathol 2002; 117 (1): 156-61
8. Uldall A, Jensen BS, Henrichsen J. Kits for the diagnosis of infectious mononucleosis compared with the Paul-Bunnell test. J Clin Chem Clin Biochem 1990; 28 (6): 423-5
9. Rogers R, Windust A, Gregory J. Evaluation of a novel dry latex preparation for demonstration of infectious mononucleosis heterophile antibody in comparison with three established tests. J Clin Microbiol 1999; 37 (1): 95-8
10. Patrascu IV, Ghenoiu O, Tache M. Epstein-Barr virus (EBV): V. Incidence of EBV antibodies in patients with rheumatic diseases. Virologie 1989; 40 (1): 25-9
11. Aalto SM, Linnavuori K, Peltola H, et al. Immunoreactivation of Epstein-Barr virus due to cytomegalovirus primary infection. J Med Virol 1998; 56 (3): 186-91
12. Deyi YM, Goubau P, Bodeus M. False-positive IgM antibody tests for cytomegalovirus in patients with acute Epstein-Barr virus infection. Eur J Clin Microbiol Infect Dis 2000; 19 (7): 557-60

13. Rhodes G, Smith RS, Rubin RE, et al. Identical IgM antibodies recognizing a glycine-alanine epitope are induced during acute infection with Epstein-Barr virus and cytomegalovirus. J Clin Lab Anal 1990; 4 (6): 456-64
14. Horwitz CA, Henle W, Henle G, et al. Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. J Infect Dis 1985; 151 (6): 1150-3
15. Fleisher GR, Collins M, Fager S. Humoral immune response in infectious mononucleosis: late emergence of anti-EA (R) and the effects of corticosteroid therapy. J Adolesc Health Care 1985; 6 (6): 424-8
16. Holmes GP, Kaplan JE, Stewart JA, et al. A cluster of patients with a chronic mononucleosis-like syndrome: is Epstein-Barr virus the cause? JAMA 1987; 257 (17): 2297-302
17. Buchwald D, Sullivan JL, Komaroff AL. Frequency of 'chronic active Epstein-Barr virus infection' in a general medical practice. JAMA 1987; 257 (17): 2303-7
18. Hellinger WC, Smith TF, Van Scoy RE, et al. Chronic fatigue syndrome and the diagnostic utility of antibody to Epstein-Barr virus early antigen. JAMA 1988; 260 (7): 971-3
19. Soto NE, Straus SE. Chronic fatigue syndrome and herpesviruses: the fading evidence. Herpes 2000; 7 (2): 46-50
20. Pitetti RD, Laus S, Wadowsky RM. Clinical evaluation of a quantitative real time polymerase chain reaction assay for diagnosis of primary Epstein-Barr virus infection in children. Pediatr Infect Dis J 2003; 22 (8): 736-9
21. Kimura H, Morita M, Yabuta Y, et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. J Clin Microbiol 1999; 37 (1): 132-6
22. Berger C, Day P, Meier G, et al. Dynamics of Epstein-Barr virus DNA levels in serum during EBV-associated disease. J Med Virol 2001; 64 (4): 505-12
23. Chan KH, Ng MH, Seto WH, et al. Epstein-Barr virus (EBV) DNA in sera of patients with primary EBV infection. J Clin Microbiol 2001; 39 (11): 4152-4
24. Brengel-Pesce K, Morand P, Schmuck A, et al. Routine use of real-time quantitative PCR for laboratory diagnosis of Epstein-Barr virus infections. J Med Virol 2002; 66 (3): 360-9
25. Gan YJ, Sullivan JL, Sixbey JW. Detection of cell-free Epstein-Barr virus DNA in serum during acute infectious mononucleosis. J Infect Dis 1994; 170 (2): 436-9
26. van Esser JW, van der HB, Meijer E, et al. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. Blood 2001; 98 (4): 972-8
27. van Esser JW, Niesters HG, Thijsen SF, et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. Br J Haematol 2001; 113 (3): 814-21
28. Test for infectious mononucleosis. BMJ 1980; 280 (6224): 1153-4
29. Andiman WA. Diagnostic procedures for viral, rickettsial and chlamydial infections. 6th ed. Washington, DC: American Public Health Association Inc., 1989
30. Niesters HG, van Esser J, Fries E, et al. Development of a real-time quantitative assay for detection of Epstein-Barr virus. J Clin Microbiol 2000; 38 (2): 712-5
31. Laroche C, Drouet EB, Brousset P, et al. Measurement by the polymerase chain reaction of the Epstein-Barr virus load in infectious mononucleosis and AIDS-related non-Hodgkin's lymphomas. J Med Virol 1995; 46 (1): 66-74
32. Kimura H, Nishikawa K, Hoshino Y, et al. Monitoring of cell-free viral DNA in primary Epstein-Barr virus infection. Med Microbiol Immunol (Berl) 2000; 188 (4): 197-202
33. Lei KI, Chan LY, Chan WY, et al. Diagnostic and prognostic implications of circulating cell-free Epstein-Barr virus DNA in natural killer/T-cell lymphoma. Clin Cancer Res 2002; 8 (1): 29-34
34. Lei KI, Chan LY, Chan WY, et al. Quantitative analysis of circulating cell-free Epstein-Barr virus (EBV) DNA levels in patients with EBV-associated lymphoid malignancies. Br J Haematol 2000; 111 (1): 239-46
35. van Kooy B, Thijsen SFT, Meijer E, et al. Sequence analysis of EBV DNA isolated from mouth washings and PBMC's of healthy individuals and blood of EBV-LPD patients. J Clin Virol 2003; 28 (1): 85-92
36. Ohshima K, Suzumiya J, Ohga S, et al. Integrated Epstein-Barr virus (EBV) and chromosomal abnormality in chronic active EBV infection. Int J Cancer 1997; 71 (6): 943-7
37. Ohshima K, Suzumiya J, Kanda M, et al. Integrated and episomal forms of Epstein-Barr virus (EBV) in EBV associated disease. Cancer Lett 1998; 122 (1-2): 43-50
38. Jones MD, Griffin BE. Clustered repeat sequences in the genome of Epstein Barr virus. Nucleic Acids Res 1983; 11 (12): 3919-37
39. Stevens SJ, Pronk I, Middeldorp JM. Toward standardization of Epstein-Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. J Clin Microbiol 2001; 39 (4): 1211-6

Correspondence and offprints: Dr *Rianne Luderer*, Diakonessenhuis, Bosboomstraat 1, Utrecht, 3582 KE, The Netherlands.
E-mail: rluderer@diakhuis.nl