Diagnostic Evaluation of a New Panel of Assays for Epstein-Barr Virus Serology

Radtke M. [1], Gärtner B. [2], Franke D. [1]

[1] medac GmbH, Diagnostic Division, Hamburg, Germany, [2] Institute of Virology, University Hospital, Homburg/Saar, Germany

Introduction

The diagnosis of primary Epstein-Barr virus (EBV) infection is based mainly on serology. It is performed by the detection of specific antibodies (IgG/IgM) to certain EBV antigens, mostly EBV nuclear antigen 1 (EBNA-1) and EBV capsid antigen complex (VCA). This study was performed to evaluate the diagnostic performance of the new commercial assays EBV EBNA-1-IgG-ELISA PKS medac, EBV VCA-IgG-ELISA PKS medac, and EBV VCA-IgM-ELA Test PKS medac using indirect immunofluorescence (IFA) as reference.

Material and Methods

150 sera from immunocompetent individuals with past EBV infection (50), acute EBV infection (50), and without infection (50, EBV negative) were investigated.
In-house IFA using P3HR1 cells for anti-VCA IgG and IgM detection and cells expressing recombinant EBNA-1 for anti-EBNA-1 IgG detection were used as reference assays.
The enzyme immunoassays (EIAs) are using purified gp125 for anti-VCA IgG and IgM detection and recombinant EBNA-1 for measuring anti-EBNA-1 IgG antibodies.
Quantitative results as arbitrary units (AU/ml) are obtained for both IgG assays by single-point quantitation (SPQ). Batch specific curve parameters together with a single calibrator are used to calculate the AU values.
The VCA-IgM-ELA Test PKS medac is a µ-capture assay using an anti-gp125 mAb-peroxidase conjugate as detection system.
Discrepant results were investigated using commercial anti-VCA and anti-EBNA-1 assays (Anti-EBV EBNA IgG ELISA, Biotest; ETI-VCA-G and ETI-EBV-M reverse, DiaSorin) as confirmatory tests.

Table 1 Diagnostic interpretation of EBV serology with medac assays

EBNA-1 IgG	VCA IgG	VCA IgM	Interpretation
-	-	-	seronegative
+	+ / ± / -	-	past infection
-	+ / ± / -	+	primary infection

All other constellations have to be interpreted as unclear. In the event of an unclear result extended differential diagnostic methods would be nessessary for interpretation.

Results

Table 2 EBV negative group (n = 50)

medac	EBNA-1 IgG	VCA IgG	VCA IgM
positive	2	1	0
borderline	0	0	0
negative	48	49	50

Table 3 EBV acute infection (n = 50)

medac	EBNA-1 IgG	VCA IgG	VCA IgM
positive	1	49	49
borderline	0	1	0
negative	49	0	1

Table 4 EBV past infection (n = 50)

medac	EBNA-1 IgG	VCA IgG	VCA IgM
positive	48	49	0
borderline	1	0	0
negative	1	1	50

Table 5 Discrepant results compared to pre-definition

Serum No.	Pre-definition	EBNA-1	VCA IgG	VCA IgM	Result
1	neg	+* / +**	-* / +**	-* / -**	past*/past**
2	neg	+ / +	+ / +	- / -	past / past
3	acute	- / ND	+ / +	- / -	? / ?
4	acute	+ / +	+ / +	+ / +	? / ?
5	past	± / +	+ / +	- / ±	? / ?
6	past	- / +	+ / -	- / -	? / ?

* medac, ** confirmatory assay, ? unclear, ND not detected

In the EBV negative group 48 sera were found negative with all three EIAs. Two were EBNA-1 positive, one of which showed also a positive reaction with the medac VCA IgG assay, while both were positive with the VCA IgG confirmatory assay. Both EBNA-1 results were also confirmed by an additional EIA (tables 2, 5). Within the panel of the sera from acute infected patients one sample revealed no VCA IgM reactivity in the medac IgM ELA Test and the confirmatory assay. A second sample was (confirmed) positive with all three assays. The EIA results of the remaining 48 samples were concordant with the interpretation of the reference assays (tables 3, 5). Out of the 50 sera from patients with past infection one was EBNA-1 negative and one EBNA-1 borderline (each not confirmed, tables 4, 5). From the remaining 48 samples one showed only EBNA-1 reactivity, which is also considered an indication of past EBV infection (see table 1). The overall agreement between IFA and EIA regarding the diagnosis of EBV infection was 96%.

Conclusions

The evaluation revealed an agreement between the EIAs and the IFA reference assays of 96%. Two of the six discrepant samples were confirmed by additional commercial EIAs. The remaining four samples showed also ambiguous results with the confirmatory assays. This diagnostic performance together with the simple handling and easy evaluation makes the medac assays very useful for routine diagnosis.

4th European Conference on Viral Diseases (ConVir 4), October 25-28, 2006, Budapest, Hungary