LAB NEWS

From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

Vol. 19 (2) Dec. 2010

Notification of Method Change for Epstein Barr Virus (EBV) Serology

The indirect fluorescent antibody (IFA) technique is the gold standard method for Epstein Barr virus (EBV) serology (1). EBV serology remains essential for differentiating **uninfected, acute primary infection and past infection.** For clinical diagnosis, three antibodies are commonly tested: IgM and IgG to EB viral capsid antigen (VCA) and IgG to EB nuclear antigen (EBNA). See **Figure** and **Table 1** for serologic patterns. Although IFA remains the gold standard, it is rarely performed in clinical laboratories since it is labor intensive, time-consuming, and requires expertise in differentiating specific from non-specific staining.

Most laboratories now use an automated enzyme-linked immunoassay (ELISA). The Clinical Virology Laboratory at Yale New Haven Hospital has been one of the few clinical laboratories still performing IFA for EBV since we considered the available ELISAs to be suboptimal (2). However, IFA testing was performed only two to three times a week and each assay took 3 hrs to complete. The growing organ transplant program at YNHH has lead to an increase in EBV serology requests and the need for a shorter time to result.

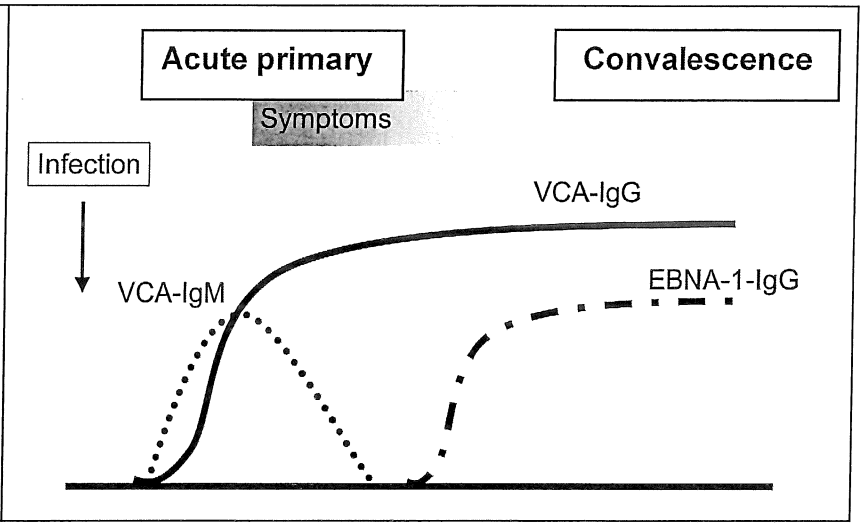
In mid-October, 2010, the Virology Laboratory transitioned from IFA to chemiluminescence immunoassay (CHLIA) using the LIAISON Analyzer (3). CHLIA has fewer non-specific reactions and a faster time to result than ELISA. The advantages of CHLIA include ability to do daily test runs during the week, with an assay time of only 1 hr. However, some differences in reactivity patterns have been noted due to the single synthetic EBV antigens used in CHLIA (instead of the multiple EBV antigens in infected lymphoma cells used for IFA), as well as the different test format. The purpose of this newsletter is to educate clinicians on these differences. See **Table 2** for more information on methods.

Figure1.

Sequential appearance of EBV VCA IgM and IgG antibodies during acute primary infection followed by EBNA-1 IgG antibody during convalescence.

While EBV VCA IgM is transient, EBV VCA IgG and EBNA IgG antibodies usually persist for the life of the host.

(Modified from B. Gartner, Reference 4)



Compared to IFA, the main differences in EBV serologic patterns observed with CHLIA are:
1) more frequent detection of only VCA-IgM early in primary infection (i.e. delayed appearance of VCA IgG compared to IFA);
2) more frequent false positive VCA-IgM reactions;
3) delayed appearance of EBNA-1 IgG during convalescence (which is actually helpful in identifying primary infections);
4) more frequent decline in EBNA-1 to negative over time, particularly in organ transplant recipients and other immunocompromised hosts, resulting in VCA IgG alone as a marker of past infection.

Thus the laboratory provides comments on test reports to aid interpretation. See **Table 1** on the next page. In addition, IFA serology will still be available, as well as EBV PCR, to resolve borderline results or special cases as needed.

Table 1. Summary and interpretation of EBV serologic patterns

CONDITION	VCA IgM	VCA IgG	EBNA-1 IgG
Typical patterns			
EBV seronegative	-	-	-
Acute EBV infection	+	+	-
Past EBV infection	-	+	+
Indeterminate pattern			
Past EBV infection, reactivation, or non-specific IgM reactivity.	+	+	+
Atypical patterns, more common with CHLIA than with IFA			
Early primary EBV infection or non-specific IgM. If indicated, submit follow-up sample in 1 week.	+	-	-
EBV seropositive, atypical pattern. Loss of detectable EBNA-1 antibody is more common in immunocompromised patients.	-	+	-

Table 2. Comparison of IFA and Chemiluminescence Antigens and Methods

EBV antibody test	IFA antigens	CHLIA antigens
Viral capsid antigen (VCA), IgM	P3HR1 Burkitt lymphoma cell line infected with replicating EBV and expressing the EBV-VCA complex of proteins	EBV VCA p18 synthetic peptide
Viral capsid antigen (VCA), IgG	Same as above	Same as above
EBV nuclear antigen (EBNA), IgG	EBV nuclear antigens expressed in the Raji Burkitt lymphoma cell line; EBNA consists of 6 proteins	EBNA-1 synthetic peptide
Method description	**IFA**	**CHLIA**
	1) Diluted patient samples are incubated with EBV infected lymphoma cells fixed on glass microscope slides to allow specific binding of anti-EBV antibodies to EBV antigens present in the cells. 2) Anti-human IgM or IgG conjugated to fluorescein is added to detect the bound EBV-antibodies. 3) The slide is examined under a fluorescent microscope.	1) Patient samples are incubated with magnetic particles coated with an EBV synthetic peptide. 2) Anti-human IgM or IgG conjugated to isoluminal is added. 3) Starter reagents are added and a flash chemiluminescence reaction is induced. 4) The light signal is measured by a photomultiplier.

For questions or concerns, call Marie L. Landry, MD, the Virology Laboratory Director at 688-3475 (marie.landry@yale.edu), or David Ferguson, the Laboratory Manager at 688-3524.

References

1. Henle W, Henle GW and Horwitz CA. Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. Hum Pathol 5:551-565, 1974.
2. Gartner BC et al. Evaluation of four commercially available Epstein-Barr virus enzyme immunoassays with an immunofluorescence assay as the reference method. Clin Diag Lab Immunol 10:78-82, 2003.
3. Feng S et al. Serological diagnosis of infectious mononucleosis by chemiluminescent immunoassay using capsid antigen p18 of Epstein-Barr virus. Clinical Chimica Acta 354:77-82, 2005.
4. Gartner BC, Epstein Barr Virus, in Manual of Clinical Microbiology, 10th edition, ASM Press (in press).