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Multicentre evaluation of the Chorus Epstein-Barr Virus test kits compared to the VCA IgM and VCA IgG immunofluorescence technique.

Ilse Nicolaï [1], Apr. Biol. B. Masschelin [2], Apr. Biol. Y. De Prest [3], Dr. Claeys [4] , Dr. S. De Raedt [5]
International Medical Products N.V /S.A [1], A.Z. Sint Jozef, Turnhout [2], Regionaal Ziekenhuis Sint Maria, Halle [3], Laboratoire Cliniques de l'Europe site Saint Michel, Bruxelles [4], Sint Elisabeth Ziekenhuis, Turnhout [5]

Introduction:

Although many ELISA kits are available for testing the presence of antibodies against Epstein-Barr virus, the immunofluorescence is in many cases still considered as being the standard technique. On different locations, the performance of the Chorus EBV kits has been tested in comparison with the immunofluorescence.
This paper is presenting a general overview of these evaluations.

Results :

Table 1: Correlation between IF VCA IgM and Chorus VCA IgM

		IF VCA IgM	
		Negative	Positive
Chorus VCA IgM	Negative	41	**7**
	Doubtful	1*	1
	Positive	/	10

* 1 sample retested on follow-up sample, giving negative results for Chorus VCA IgM and EA IgM

Additional information on 4 of those 7 samples:

IF IgG	*VCA IgG*	*EA IgG*	*EBNA IgG*
4 +	4 +	1 N	1 N 2 P & 1 Dbt

⇒ meaning, 2 past infections, 1 recovery and 1 late- or non-EBNA responder

The other 3 samples were at forehand considered as "possible false reactive on IF"
⇒ 1 sample resulted EBNA IgG negative (no-immunity or non-EBNA responder)
⇒ 2 samples resulted EBNA IgG positive, indicating a past infection

All IF VCA IgM negative samples did result negative on the Chorus VCA IgM, showing a 100% correlation between both methods.
From the 18 positive IF VCA IgM samples, only 10 did give a positive and 1 doubtful result on the Chorus VCA IgM (correlation of 11/18 or 61,1%). The remaining 7 samples, did yield a negative result on the Chorus VCA IgM, which at first sight could be considered as the Chorus VCA IgM being less sensitive. Nevertheless, from those samples, additional testing has been proven the Chorus VCA IgM is not less sensitive, but more specific. Some of those samples have been further investigated in order to confirm cross-reactivity of another virus on the IF VCA IgM (see *Brief evaluation on possible cross-reactivity, on the Chorus EBV VCA IgM*).

Table 2: Correlation between IF VCA IgG and Chorus VCA IgG

		IF VCA IgG	
		Negative	Positive
Chorus VCA IgG	Negative	7	**6**
	Doubtful	/	1
	Positive	/	45

- 2 negative VCA IgM, VCA IgG, EA IgM, EA IgG and EBNA IgG ⇒ no immunity
- 1 positive VCA IgM, negative EBNA IgG ⇒ start primo infection ?
- 2 positive VCA IgM & EA IgM, negative EA IgG and EBNA IgG ⇒ begin of primo infection
- 1 positive VCA IgM, EA IgM & EA IgG with negative EBNA IgG ⇒ end acute phase of primo infection

Again, all negative IF VCA IgG did result negative on the Chorus VCA IgG (correlation of 100%).
Although from the 52 positive IF VCA IgG, only 45 resulted positive and 1 doubtful on the Chorus VCA IgG (correlation of 46/52 or 88,5%). The other 6 samples resulted negative on the Chorus VCA IgG, where additional testing could prove that 2 samples did show a 'no-immunity' pattern, 1 sample a possible start of a primo infection, 1 defined start of a primo infection and 1 end of primo infection with a less sensitive VCA IgG. In general, one could state that the VCA IgG is more specific than the IF VCA IgG and that the obtained results correlate well with the status of the infection or the absence of an EBV infection.

Figure 1: Index found on negative, doubtful and positive samples with the Chorus EBV VCA IgM and VCA IgG

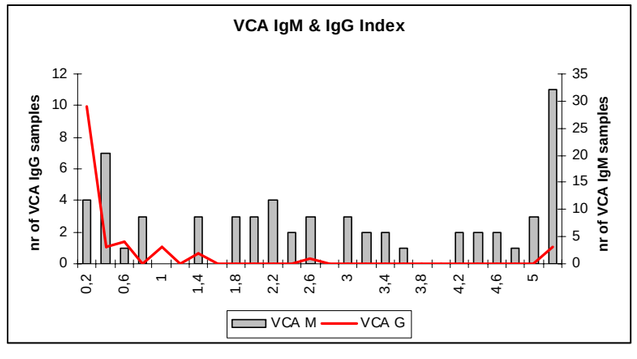

Table 3a: Correlation based on diagnosis using Immunofluorescence technique: acute primo infection or recovery primo infection.

IMMUNOFLUORESCENCE		CHORUS				
VCA IgM (n=13)	**VCA IgG** (n=13)	**VCA IgM** (n=13)	**VCA IgG** (n=13)	**EA IgM** (n=6)	**EA IgG** (n=6)	**EBNA IgG** (n=13)
All positive	All positive	- 3 positive - 1 positive* - 4 positive**	- 3 positive - 1 doubtful* - 4 negative**	- 1 positive / - 3 positive**	- 1 positive / - 1 positive** & 2 negative**	- 3 negative - 1 negative* - 4 negative**
		- 1 doubtful**	- 1 positive**	- 1 negative**	- 1 positive**	- 1 negative**
		- 4 negative***	- 4 positive***	- 1 negative***	- 1 negative***	- 1 negative, 2 positive & 1 doubtful***

Based on the positive reactivity of as well the Chorus VCA IgM as the VCA IgG, only 3/13 samples do correlate with the IF results, suggesting an acute primo infection.
* The VCA IgM positive with an VCA IgG doubtful result, is also suggesting an acute primo infection (EBNA IgG negative).
** From the 4 positive VCA IgM with negative VCA IgG and EBNA IgG reaction, the presence of EA IgG is indicating the end of an acute primo infection, while the absence of EA IgG (with positive EA IgM) is indicating the start of a primo infection. Also the doubtful VCA IgM with positive VCA IgG & EA IgG and EBNA IgG, is indicating the end of an acute primo infection.
*** The remaining 4 samples, yielding a negative reactivity on the Chorus VCA IgM while being positive on the IF VCA IgM, could be considered as a non-specific reaction on the IF VCA IgM. In 3 cases the positive/doubtful EBNA IgG is indicating a past infection and the 1 sample with EBNA IgG result could be considered as a non-EBNA or late-EBNA responder.

Table 3b: Correlation based on diagnosis using Immunofluorescence technique: no immunity (100% correlation)

IMMUNOFLUORESCENCE		CHORUS		
VCA IgM	**VCA IgG**	**VCA IgM**	**VCA IgG**	**EBNA IgG**
All negative	All negative	- 6 Negative - 1 Doubtful	All negative	All negative

Table 3c: Correlation based on diagnosis using Immunofluorescence technique: past infection

IMMUNOFLUORESCENCE		CHORUS				
VCA IgM (n=34)	**VCA IgG** (n=34)	**VCA IgM** (n=34)	**VCA IgG** (n=34)	**EA IgM** (n=13)	**EA IgG** (n=13)	**EBNA IgG** (n=34)
All negative	All positive	- 31 negative - 1 negative - 2 negative	- 31 positive - 1 positive - 2 negative	- 10 negative & 1 doubtful / - 2 negative	- 11 negative / - 2 negative	- 31 positive - 1 negative - 2 negative

Based on the Chorus results, 32/34 samples (94,1%) could confirm the diagnosis made with the IF results.
The total absence of antibodies in the 2 remaining samples tested on the Chorus panel, indicate no-immunity to EBV, while for these samples the presence of IF VCA IgG did. After retesting 1 of the VCA IgG samples, the reactivity has been defined as non-specific.

Brief evaluation on possible cross-reactivity, interferences on the Chorus EBV VCA IgM.
One centre did test 5 positive and 1 doubtful CMV IgM and 2 positive HCV samples on the Chorus VCA IgM, yielding 6 negative and 1 doubtful result. Three samples with high total IgM concentration and 3 samples with a polyclonal γ-fraction on electrophoresis, did not interfere with the Chorus VCA IgM test.

Conclusion:

Individual comparison of both VCA IgM and VCA IgG kits, could suggest a lower sensitivity (61,1% IgM, 88,5% IgG) of the Chorus kits. When looking at the combination of VCA IgM and IgG, the Chorus kits correlate much better with the clinical diagnosis. In general the Chorus VCA IgM and VCA IgG kits are more specific than the IF VCA IgM and IF VCA IgG kits, showing until now no cross-reactivity with other agents. Even with a high specificity, the analytical sensitivity has been proven to be high enough to capture the specific antibodies during seroconversion, acute infection, recovery and state of immunity. Not only the objective aspect of reporting results, but also the performance of the Chorus EBV test kits can be considered as an excellent alternative for the immunofluorescence technique.

International Medical Products s.a./n.v., Chaussée de la Hulpe 181/21 – Terhulpsesteenweg 181/21, 1170 Bruxells – Brussel, Tel: +32(0)2 660 50 75 – Fax: +32(0)2 660 20 98 – E-mail: info@intermed.be