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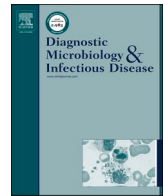
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## Letter to the Editor



## Results of a French external quality assessment program for Epstein Barr virus serology

Primary Epstein-Barr virus (EBV) infection is associated with infectious mononucleosis (IM), and its persistence is associated with 1-2 % of cancers worldwide and immune-mediated inflammatory diseases such as multiple sclerosis [1]. EBV serology is widely used to diagnose IM and to determine EBV serological status in donors and transplant recipients [2,3]. Given the wide variety of EBV serological assays, external proficiency testing is needed to control the analytical performance of laboratories for the serological diagnosis of EBV-associated diseases.

Under the supervision of the French National Agency for the Safety of Medicines and Health Products (ANSM), we conducted an external quality assessment (EQA) for the detection of anti-EBV antibodies involving 180 French laboratories. The EQA panel consisted of two serum samples with variable reactivity to EBV for the analysis of anti-VCA (EA) IgG, anti-VCA IgM and anti-EBNA-1 IgG antibodies. The samples were prepared from a pool of serum according to standard operating procedures (Cerba Xpert). Prior to shipment, the samples were characterized at a reference laboratory for EBV diagnosis, the Grenoble University Hospital (France). Each laboratory received a 1 mL blind aliquot of each sample in November 2021. Each sample shipment was accompanied by a cover letter with basic instructions and a reporting form. Participants were asked to use their regular routine procedures for the determination of anti-EBV antibodies.

The study showed a heterogeneous landscape of anti-EBV serological testing among the participating laboratories. The most commonly used commercial immunoassays were Biomerieux Vidas® (n = 65), Diasorin Liaison® (n = 44), Abbott Architect® (n = 26) and Abbott Alinity® (n = 24). Overall, the results of this EQA for EBV serology showed an

excellent agreement between the participating laboratories for the diagnosis of past and primary infection (98 % and 97 %, respectively). However, detailed analysis suggests that there is room for further improvement. The most discrepant results were related to the determination of anti-VCA IgG antibodies in sample 2, which mimicked a primary infection profile (Table 1). This sample showed a low level of anti-VCA IgG antibodies (Fig. 1). Eleven participating laboratories, particularly those using the Liaison assay, failed to detect anti-VCA IgG antibodies in sample 2. Three of them did not conclude a primary infection profile, although all of them detected anti-VCA IgM antibodies in this sample.

Serological profiles with isolated anti-VCA IgM or combined anti-VCA IgM and IgG antibodies (without EBNA IgG) are suggestive of primary infection, but isolated IgM antibodies may also be associated with false-positive results (cross-reaction, non-specific immune response) [4, 5]. Clinical data, serological follow-up or further diagnostic approaches may help to distinguish false-positive IgM marker results from early primary infection [3,6]. However, delayed detection of IgG antibodies after primary infection could be subject to misinterpretation in laboratories, as we observed in our study with the Liaison® assay. Optimizing the cut-off value of the Liaison® VCA IgG marker could facilitate the interpretation of serological results relating to cases of primary infection or the diagnosis of past infection, where the marker may also lack sensitivity, as previously reported [6,7].

Table 1

Results of the EQA scheme for EBV serology.

	Expected result	N. of discrepant results for the assay <sup>a</sup>				Total
		Architect N=26	Alinity N=24	Liaison N=44	VIDAS N=64	
Sample 1: Past infection	VCA IgM negative	0	0	0	2	2
	VCA/EA IgG positive	0	0	0	0	0
	EBNA IgG positive	0	0	1	2	3
	Interpretation <sup>c</sup>	0	0	1	2	3
Sample 2: Primary-infection	VCA IgM positive	0	0	0	2	2
	VCA/EA IgG positive	0	0	10 <sup>b</sup>	0	10
	EBNA IgG negative	0	0	1	2	3
	Interpretation <sup>c</sup>	0	0	3	2	5

<sup>a</sup> others immunoassays (n=22) included Elecsys n=6; Alegria n=4; Isys n=3; Bioplex n=2; Imulite n=2; Virclia n=2; Euroimmun ELISA n=1; Vircell Orgentec Virapid mono M/G n=1; reagent not documented n=1. The Vircell Orgentec test gave one false negative for the VCA IgG marker in sample 2 but the laboratory concluded correctly.

<sup>b</sup> discrepant results included 7 equivocal and 3 negative values

<sup>c</sup> The 8 misinterpretations came from 5 laboratories. Among them, two laboratories using VIDAS assay were suspected of having inverted samples 1 and 2.

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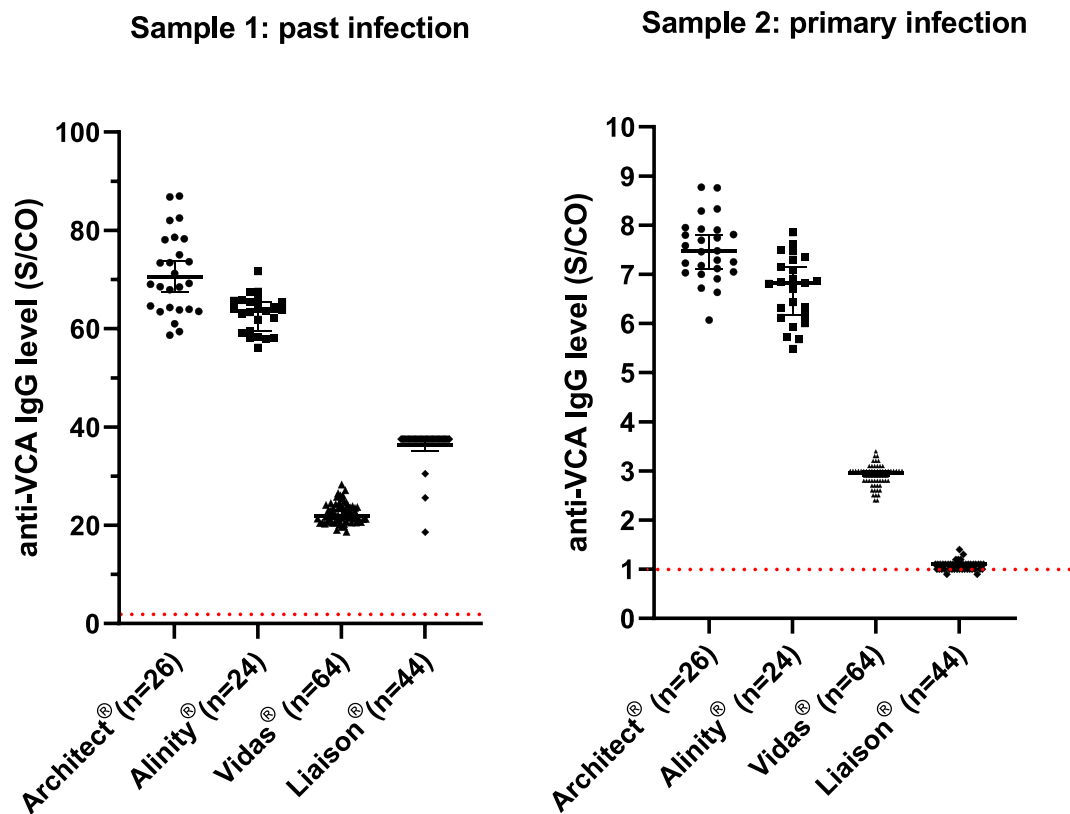


Fig. 1. Anti-VCA (EA) IgG level in sample 1 (past infection, left) and sample 2 (primary infection, right) according to the EBV immunoassay used by the participating laboratories. Anti-VCA IgG values of VIDAS and Liaison assays were converted in Signal / Cut Off (S/CO) which corresponds to the values of antibody level provided by the automated assay divided by the threshold of the assay. The positivity threshold of each assay was therefore set at  $\geq 1$  for all the represented assays (dotted line).

#### CRediT authorship contribution statement

**Julien Lupo:** Formal analysis, Methodology, Validation, Writing – original draft, Supervision. **Aurélien Truffot:** Writing – review & editing. **Raphaële Germe:** Writing – review & editing. **Anne-Karen Faure:** Writing – review & editing. **Patrice Morand:** Writing – review & editing. **Elizabeth Francois-Burg:** Data curation, Formal analysis, Project administration.

#### Declaration of Competing Interest

The authors declare no conflicts of interest.

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