VirClia® Epstein-Barr Virus Viral Capside Antigen (VCA) IgG, VCA IgM and EBV Nuclear Antigen (EBNA) IgG evaluation compared to Diasorin EBV

Introduction

Primary infection with the Epstein-Barr virus (EBV) is the primary causative factor in the development of infectious mononucleosis (IM). Following initial infection, EBV remains in a state of persistent latency within B lymphocytes. However, persistent presence of EBV may also be associated with various malignancies such as Burkitt lymphoma, post-transplantation lymphoproliferative disease, and nasopharyngeal carcinoma. Globally, more than 95% of adults are infected with EBV, making EBV serology crucial for proper clinical management of IM, EBV-associated cancers, and determining serological status in pretransplant patients.

For qualitative detection of EBV-specific antibodies in immunocompetent individuals, three serological parameters are essential: VCA IgG, VCA IgM, and EBNA IgG. Viral Capsid antigens generate lifelong persistent IgG titers while IgM antibodies are transiently produced. The presence of VCA IgM antibodies does not necessarily precede VCA IgG presentation; both antibody classes can appear simultaneously. In contrast, late in the course of infection, EBNA IgG antibodies are produced and persist lifelong. Serology for detecting these specific antibodies is typically performed using a single acute-phase serum sample and allows for stage-specific diagnosis as Acute Infection, Past Infection or Recent Past infection.

In this internal retrospective study conducted by Vircell, the correlation between their VirClia® assays for Epstein-Barr Virus VCA IgG, VCA IgM and EBNA IgG was evaluated using a sample panel previously characterized with Liaison Diasorin EBV serological markers. Additionally, complementary assays such as Heterophile antibodies to the Paul-Bunnell Antigen and a VCA IgG avidity test were performed to assess and classify the patient samples' EBV infection status as Acute EBV, Past EBV, or Recent Past EBV. The results obtained from Vircell' VirClia® and Diasorin' Liaison EBV serological markers were compared to this patient classification status.

Materials and methods

A total of 142 serum samples were included in this study. These samples were obtained from patients who were referred for EBV diagnostic purposes. The evaluation of these samples was conducted using three different assays: VIRCLIA® EPSTEIN-BARR VCA IgG, VIRCLIA® EPSTEIN-BARR VCA IgM, and VIRCLIA® EPSTEIN-BARR EBNA IgG MONOTEST.

Prior to this study, all the serum samples had already been tested and characterized for the presence of VCA IgG, VCA IgM, and EBNA IgG antibodies using the Liaison Diasorin EBV assays. Additionally, information regarding patient age and gender was available for each sample.

Samples were further investigated for Heterophile antibodies and the VCA IgG antibody avidity as a complementary EBV markers for assessing the patient serological status, in order to analyse the individual performance of each of the VirClia® and Liaison EBV markers in these patient groups.

The Heterophile antibodies are present in sera of IM patients and react to erythrocytes of other animal species. These antibodies, not specific for the EBV antigens, are present in 80-85% of the adult and adolescent patients and peak in the first month of infection. Its detection has a high diagnostic value for the acute phase of EBV infection. Presence of heterophile antibodies was determined by utilizing the INFECTIOUS MONONUCLEOSIS HETEROPHILE VIRCLIA® IgM MONOTEST.

The VCA IgG antibody avidity was determined by utilizing an *In House* method in all samples showing positive result with the EPSTEIN-BARR VCA VIRCLIA® IgG MONOTEST. The sample dilution protocol on the EPSTEIN-BARR VCA VIRCLIA® IgG MONOTEST was modified. Samples have been tested for VCA IgG presence with and without urea as chaotropic agent in the sample diluent of the monotest strips. The urea causes the low-affinity antibodies to be removed from the solid phase, while high-affinity antibodies remain bound to their specific antigens in the solid phase of the Monotest strips. An Antibody avidity Index was determined by comparing reactivity of the two VirClia® VCA IgG assays, for the same patient sample, with and without the avidity reagent. Samples have been classified as a Low avidity, compatible with a recent primo-infection of less than 4 months, Intermediate avidity, and high avidity compatible with past-infection of more than 4 months approximately.

All samples were also tested for CMV IgM presence with the CYTOMEGALOVIRUS VIRCLIA® IgM MONOTEST in order to disregard a possible cross-reaction that could be compatible with a non-specific reaction of the EBV serology markers. Additionally, a CMV IgG Avidity test was performed in all the CMV IgM positive samples to assess the trueness of the CMV infection with the CYTOMEGALOVIRUS VIRCLIA® IgG AVIDITY MONOTEST.

Correlation of the VirClia® EBV serological markers is calculated by utilizing the Liaison Diasorin results as a reference. Additionally, the EBV infectious status of each of the samples has been stablished based on the results of the three EBV serological markers, VCA IgG, VCA IgM and EBNA IgG, along with a coherent and compatible result of the Heterophile antibody and VCA IgG avidity test. The defined EBV status were as follows: “Acute EBV,” “Recent Past EBV,” “Past EBV” and “Not interpretable.” Other possible serological status was “Acute CMV” when an active CMV infection could be the reason for the reactivity of the EBV markers.

The Vircell and the Liaison Diasorin EBV individual serological markers results have been compared to this patient classification status to assess their performance.

Results

A total of 142 patient samples suspected for an Infectious Mononucleosis infection patient and characterized for VCA IgG, VCM IgM and EBNA IgG from Liaison Diasorin were analysed with the corresponding VirClia® EBV assays.

Correlation analysis

For VCA IgG results, out of the 142 samples, 121 were positive and 5 were negative for both Liaison VCA IgG or VirClia® VCA IgG. Three samples showed positive results with Diasorin but indeterminate results with VirClia®, while 13 samples tested positive with Liaison but negative with VirClia®. Refer to Table 1 for detailed results.

		VIRCLIA		
VCA IgG		Positive	IND	Negative
LIAISON	Positive	121	3	13
	IND	0	0	0
	Negative	0	0	5

Table 1: Liaison and VirClia® results for VCA IgG samples

The correlation between VirClia® VCA IgG and Liaison VCA IgG was calculated as 88.7% (126/142). If the indeterminate results from VirClia® VCA IgG are excluded, the correlation increases to 90.7% (126/139).

For VCA IgM results, out of the 142 samples, both Liaison VCA IgM and VirClia® VCA IgM assays yielded positive results in 109 cases. Additionally, there were 29 samples that tested positive with Diasorin but negative with VirClia®, and four samples that showed positive results with Liaison but indeterminate results with VirClia®. Refer to Table 2 for detailed results.

		VIRCLIA		
VCA IgM		Positive	IND	Negative
LIAISON	Positive	109	4	29
	IND	0	0	0
	Negative	0	0	0

Table 2: Liaison and VirClia® results for VCA IgM samples

The correlation between VirClia® VCA IgM and Liaison VCA IgM was determined as 76.8% (109/142). Excluding the indeterminate results from VirClia®, the correlation becomes 79.0% (109/138) when compared to Liaison's test.

For the EBNA IgG results, out of the 142 samples, 53 were positive and 41 were negative for both Liaison EBNA IgG and VirClia® EBNA IgG. 5 samples were positive by Diasorin and negative by VirClia® and 22 samples were positive with VirClia® and negative Liaison. 6 samples were indeterminate by VirClia® and 12 samples were indeterminate by Liaison. Results can be seen in Table 3.

		VIRCLIA		
EBNA IgG		Positive	IND	Negative
LIAISON	Positive	53	2	5
	IND	8	0	4
	Negative	22	4	41

Table 3: Liaison and VirClia® results for EBNA IgG samples

The correlation VirClia® EBNA IgG vs. Liaison VCA EBNA was calculated as 67.6% (94/139). If the VirClia® EBNA IgG Indeterminate results are not considered, correlation with Liaison EBNA IgG increases to 74.0% (94/127).

Agreement compared to EBV serological status

The EBV infection status of each sample was determined using the three EBV serological markers: VCA IgG, VCA IgM, and EBNA IgG, in conjunction with a consistent and compatible outcome from the Heterophile antibody and VCA IgG avidity test results. The following classifications have been defined: Acute EBV, Past EBV, and Recent Past EBV. A comprehensive overview of different combinations of EBV serological markers is provided in Table 4.

	VCA IgM	VCA IgG	EBNA IgG	Heterophile	VCA IgG avidity
Acute EBV	+	+ or -	-	+	Low
Past EBV	-	+	+	-	High
Recent Past EBV	+ or -	+	+ or -	-	Intermediate/Low

Table 4: EBV patient status based on the serological markers.

Based on the specified criteria, out of the 142 patient samples analysed, 70 have been classified as Acute EBV infections, 56 as Past EBV infections, and 7 as Recent Past EBV infections. Furthermore, two samples have been identified as Acute CMV infections, primarily due to their positive CMV IgM results coupled with a low avidity CMV IgG, suggesting a potential cross-reaction with EBV. Lastly, seven samples remain classified as Non-interpretable, owing to inconclusive results or by the unavailability of the VCA IgG Avidity test.

Acute EBV results

Out of the 70 samples classified as ACUTE EBV, Liaison VCA IgM detected all 70 samples as positive, while VirClia® VCA IgM detected 69 out of 70 samples as positive. However, there were discrepancies in the results for EBNA IgG marker. Liaison reported 4 samples as EBNA IgG positive, whereas VirClia® reported 12 samples as EBNA IgG positive. These results were unexpected since the awaited result for these patients would be EBNA IgG negative. Additionally, a positive EBNA IgG result in these patients did not match with the positive result of Heterophile antibody and the Low avidity for VCA IgG antibodies.

Past EBV results

From the 56 samples classified as PAST EBV, Liaison reported all 56 samples as VCA IgM positive and only 12 samples as EBNA IgG negative. The expected results would be VCA IgM negative and EBNA IgG positive. On the other hand, VirClia® reported only 28 out of 56 results as VCM IgM positive and just one sample mismatched with an EBNA IgG negative result. In Past EBV patients, the VCA IgM positive and the EBNA IgG negative results do not correspond to the negative result on Heterophile antibody and High avidity for VCA IgG antibodies, that have been reported for those samples.

Recent Past EBV results

From the seven samples classified as RECENT PAST EBV, both Liaison and VirClia® report compatible results for the three EBV VCA IgM, VCA IgG and EBNA IgG serological markers. All those seven samples have Low or intermediate VCA IgG Avidity and a negative result for heterophile antibodies.

CMV infection

Two patient samples showed a positive result for CMV IgM and low avidity for CMV IgG, which is consistent

with an active CMV infection. This active CMV infection may produce a possible cross-reaction between common CMV and EBV antigens.

Non-Interpretable

Seven patient samples showed incoherent pattern of EBV serological markers along the unavailability of the VCA IgG avidity test to solve discrepancies. All those patients were considered non-Interpretable.

Discussion

In this internal retrospective study Vircell has evaluated the VirClia® Epstein-Barr Virus VCA IgG, VCA IgM and EBNA IgG immunoassays with a panel of samples that have been previously characterized with the Liaison Diasorin EBV serological markers.

Apart from assessing the strict correlation between each of the three EBV markers, Vircell aimed to compare the results obtained from both VirClia® and Liaison assays in relation to an EBV serological status. However, classifying the serological status of EBV infection solely based on three serological markers can be challenging if discrepant results arise when different methodologies are used. To address this issue, additional complementary assays were performed on all samples.

The Heterophile antibodies assay to the Paul-Bunell antigen and the VCA IgG Avidity test were carried out as complementary tests to determine and classify the EBV infection status of each patient sample. The presence or absence of Heterophile antibodies indicates whether an acute or past EBV infection is present in adults and adolescents. Reactivity to Paul-Bunell antigen may be lower in children. Additionally, low VCA IgG avidity is expected in acute infections while high avidity is indicative of past infections. In cases where recent past infections occur, Heterophile antibodies should be negative and VCA IgG avidity may range from intermediate to high.

The EBV infection status of each sample has been definitively determined by assessing three EBV serological markers: VCA IgG, VCA IgM, and EBNA IgG. Furthermore, these results have been corroborated with the Heterophile antibody and VCA IgG avidity test to yield a reference EBV status result. Consequently, the samples have been categorized into three distinct groups: Acute EBV, Past EBV, and Recent Past EBV.

It is important to consider that common infections caused by other Herpes viruses, such as Cytomegalovirus (CMV), can lead to potential cross-reactions with the EBV because of sharing common sequences in their antigenic proteins. To address this potential interference, CMV IgM determination has been performed to all patient samples. Moreover, CMV IgG avidity test has been also performed to all samples reporting a CMV IgM positive result, to assess the specificity or trueness of this IgM result. The CMV IgM positive result should be compatible with a low avidity CMV IgG, as it happened in two samples that have been characterized as Acute CMV. Finally, a serological profile not compatible with acute EBV, past EBV, recent past EBV or acute CMV has been classified as Non-Interpretable. Seven samples enter this category.

The results obtained from the three EBV serological markers using VirClia® and Liaison demonstrate a significant degree of correlation, ranging from 67% to 88.7%. This correlation increases to 74% to 90.6% when the doubtful VirClia® results are excluded. Notably, VCA IgG exhibited the highest correlation, accounting for 89% to 90.65% of the total results.

The comparatively lower correlation observed with the other two serological markers, VCA IgM and EBNA IgG, is better explained when the serological status is examined in depth.

Acute EBV

Among the 70 samples classified as Acute EBV, all of them tested positive for Liaison VCA IgM. VirClia® VCA IgM also reported all of these samples as positive for VCA IgM marker, except for one sample. However, there were discrepancies in the results for EBNA IgG between the two assays. The Liaison assay reported 4 samples as EBNA IgG positive, while the VirClia® assay reported 12 samples as EBNA IgG positive. In cases of acute EBV infection, it is expected to have a negative result for EBNA IgG. These findings suggest that VirClia® and Liaison have similar sensitivity in detecting VCA IgM in acute EBV samples. However, the VirClia® assay appears to be more sensitive than the Liaison assay when it comes to detecting EBNA IgG in this type of samples.

Past EBV

Surprisingly, all 56 samples classified as Past EBV were reported as VCA IgM positive by the Liaison assay. However, only half of these samples were positive by VirClia® VCA IgM. The expected result for an EBV past infection would be a VCA IgM absence, which is compatible with the VCA IgG high avidity and negative results to Heterophile antibodies (with a three borderline exceptions) reported by all the 56 samples. These results indicate that the Liaison VCA IgM may be less specific or excessively sensitive compared to VirClia® VCA IgM in past EBV cases.

Furthermore, while the Liaison assay reported 12 samples as negative for EBNA IgG, only one sample was reported as negative by the VirClia® assay. In cases of past EBV infection, a positive result is expected for EBNA IgG. This suggests that the Liaison assay may be less sensitive than VirClia® in detecting this marker in past infections.

Recent Past EBV

In terms of Recent past EBV, both the Liaison and VirClia® assays reported compatible results for the three EBV serological markers (VCA IgM, VCA IgG, and EBNA IgG) in all 7 samples. These samples showed low or intermediate VCA IgG avidity and tested negative for Heterophile antibodies.

Overall, the correlation between the VirClia® and Liaison EBV serological markers satisfactory, with notable strong correlation in the case of the VCA IgG assay. However, when scrutinized the results of Liaison and VirClia® assays considering the established EBV patient status, certain discrepancies appear. It is plausible that slight disparities in assay adjustments for VCA IgM and EBNA IgG between Liaison and VirClia® concerning sensitivity and specificity, may contribute to mismatches, particularly in the classification of Acute and Past EBV infections.

The Liaison VCA IgM assay exhibits a similar level of sensitivity compared to VirClia® when assessing patients with Acute EBV infections. However, it demonstrates lower specificity in comparison to VirClia®, particularly in cases involving patients with Past EBV infections. Notably, the Liaison VCA IgM assay tends to detect the presence of VCA IgM antibodies in a higher number of cases compared to VirClia®. This phenomenon suggests a potential excess of sensitivity, particularly in samples concurrently displaying a high avidity for VCA IgG antibodies.

Furthermore, the Liaison EBNA IgG assay appears to be less sensitive in cases of Past EBV infections, whereas the VirClia® EBNA IgG assay demonstrates more sensitivity when evaluating patients with Acute EBV infections.

The results obtained in this study by Vircell indicate that the performance of both the VirClia® and Liaison assays is comparable, while acknowledging the inherent differences that can arise among different in-vitro diagnostic manufacturers. Consequently, both the VirClia® and Liaison assays demonstrate suitability for routine use in clinical laboratories, particularly in the serological diagnosis of EBV infection.

This study may have some limitations associated with the availability of negative samples. Given the high prevalence of EBV infection in the adult population, obtaining completely negative EBV samples proved to be challenging, thereby affecting the ability to thoroughly evaluate the correlation between VirClia® EBV and Liaison EBV serological markers.

It is worth emphasizing the valuable role played by Heterophile antibody detection and VCA IgG avidity testing as complementary tools for the accurate evaluation of EBV status. Notably, EBV serological markers, including VCA IgM, VCA IgG, and EBNA IgG, produced by different manufacturers may exhibit variations in the sensitivity and specificity balance, which can introduce a degree of inaccuracy. Relying on a single serological marker result in isolation can yield an erroneous conclusion regarding EBV serological status. Furthermore, even the combination of the three primary EBV serological markers from a single manufacturer may also result in an incorrect assessment.

The incorporation of additional assays such as Heterophile antibody detection and VCA IgG avidity testing significantly enhances the assessment of compatibility and consistency with the results derived from conventional EBV markers, ultimately contributing to a more accurate diagnosis.

Heterophile antibody detection is a method than can be run automatically on VirClia® platforms with the existing INFECTIOUS MONONUCLEOSIS HETEROPHILE

VIRCLIA® IgM MONOTEST. Worth to mention that the Heterophile antibody assay is a serological marker not present in the Liaison EBV portfolio, hence this marker should be tested manually by any of the rapid test commercially available, including the Vircell Virapid Mono. The Vircell VirClia® is the only platform, along Biorad Bioplex, having an heterophile antibody assay able to be run automatically in a continuous loading and random-access mode. Vircell highly recommends to incorporate the Heterophile antibody assay as a part of the EBV diagnostic algorithm on laboratories running the VirClia® EBV markers.

The VCA IgG Avidity test utilized in this study is an existing *In-House* assay in Vircell, not commercially available. Its excellent performance in this study, mean that Vircell has decided to register the VCA IgG Avidity it as a new commercial IVD assay in the VirClia® portfolio in the near future.

Conclusion

Correlation of VirClia® EBV serological markers with Liaison EBV markers VCA IgM, VCA IgG and EBNA IgG is correct and particularly strong with the VCA IgG assay. The lower correlation observed with the VCA IgM and EBNA IgG assay is better explained when considering the serological status of the samples. The EBV infection status of each patient sample has been determined by assessing the three EBV serological markers in combination with Heterophile antibody detection and the VCA IgG avidity test, to categorize the samples into three distinct groups: Acute EBV, Past EBV, and Recent Past EBV.

The results from Liaison VCA IgG show a correlation of approximately 90% with VirClia® VCA IgG results. When comparing Liaison VCA IgG results in both Acute and Past EBV infections to those provided by VirClia® VCA IgG, they are found to be very similar.

Liaison VCA IgM demonstrates equal sensitivity to VirClia® IgM in Acute EBV patients but tends to be less specific than VirClia® VCA IgM in Past EBV patients. This phenomenon leads to a higher number of positive reactions in samples that clearly indicate a Past EBV infection status characterized by high avidity VCA IgG antibodies and negative results for Heterophile antibodies.

In contrast, Liaison EBNA IgG appears to be less sensitive in cases of Past EBV infections compared to VirClia® EBNA IgG, which seems more sensitive in cases of Acute EBV infections.

Based on the findings from this study, it can be concluded that both VirClia® and Liaison demonstrated satisfactory performance, when assessing the EBV serological status. Consequently, they can be effectively integrated into routine laboratory procedures for the serological diagnosis of EBV infection.

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