

Evaluation of a new CLIA Monotest Assay for the Detection of *Helicobacter pylori* Antigen: A Retrospective comparison with a Liaison® and SD Biosensor assays

Introduction

Helicobacter pylori is a bacterium found in the gastric mucosa of the human stomach and is associated with various digestive diseases. Infection with *H. pylori* is a well-established cause of gastric and duodenal ulcers, as well as a risk factor for gastric carcinoma and lymphoma. The detection of *H. pylori* antigen in stool is a reliable method used to aid in the diagnosis of infections in untreated patients.

In this internal retrospective study Vircell has evaluated the new HELICOBACTER PYLORI Ag VIRCLIA® MONOTEST and the *H Pylori Ag* FIA assay from SD Biosensor with a panel of samples that have been previously characterized with the Liaison® Meridian *H. pylori* SA from Diasorin.

Apart from assessing the strict correlation between each of the three manufacturers, Vircell also aimed to compare the performance in terms of Sensitivity and Specificity obtained for VirClia®, SD Biosensor and Liaison® assays in relation to a consensus result. The Premier Platinum HpSA PLUS ELISA assay from Meridian has been used to test the discrepant samples observed among the three different assays utilized: VirClia®, Liaison® and SD Biosensor, in order to build a consensus, result to better evaluate the performance of each assay.

Materials and methods

A total of 313 stool samples were included in this study. These samples were obtained from patients who were referred for *Helicobacter pylori* Antigen diagnostic purposes. Prior to this study, all the stool samples had already been tested and characterized for the presence of *Helicobacter pylori* Antigen using the CLIA assay Liaison® Meridian *H. pylori* SA from Diasorin.

The evaluation of these samples was conducted using a chemiluminescence based assay, the HELICOBACTER PYLORI Ag VIRCLIA® MONOTEST. Additionally, all the samples were further evaluated for *Helicobacter pylori* Antigen by using the *H Pylori Ag* FIA from SD Biosensor, which is a Lateral Flow assays based on fluorescence reading.

Two independent VirClia® assay agreement studies in front of the Diasorin and the SD Biosensor assays have been performed. The relative sensitivity, relative specificity and percentage agreement versus these two assays considered as a predicate reference have been calculated.

Additionally, all discrepant samples between the three different assays: VirClia®, Liaison® and SD Biosensor were also evaluated with the Premier Platinum HpSA PLUS ELISA from Meridian Bioscience to establish a consensus result. For that purpose, ELISA Meridian result from the same sample should match with the results with the other assays utilized to build the consensus.

Three different consensus results have been considered based on a same sample result, positive or negative, for each of the following manufacturer's combination:

- . Meridian – Liaison® – SD Biosensor
- . Meridian – VirClia® – SD Biosensor
- . Meridian – VirClia® – Liaison®

In these three analyses, the results of one of the three assays evaluated, VirClia®, Liaison® and SD Biosensor were excluded from the consensus result to avoid incorporating bias. Indeed, including all the assays in the consensus result definition would improve the performance of all the test.

Therefore, the performance of the VirClia® assay has been determined against a consensus result based on Liaison®, SD Biosensor and Meridian results. Performance of the Liaison® assay has been determined against a consensus result based on VirClia®, SD Biosensor and Meridian and finally, the Performance of the SD Biosensor assay has been determined against a consensus result based on VirClia®, Liaison® and Meridian results.

The results between each of the three methods with the consensus result were also compared through Cohen’s kappa coefficient. (k). Additionally, the Diagnostic performance of each of the three assays against a consensus was evaluated through the Area under the curve (AUC) from a receiver operating characteristic (ROC) curve.

Results

A total of 313 patient samples suspected for an active *Helicobacter pylori* infection and characterized for *Helicobacter pylori* Antigen from Liaison® Diasorin were analysed with the corresponding VirClia® and SD Biosensor assays.

Out of the 313 samples, 39 samples reported discrepant results from either of the VirClia®, Liaison® and SD-Biosensor assays. Those discrepant samples were then evaluated with the Premier Platinum HpSA PLUS ELISA assay from Meridian to build a consensus result from three independent assays: Meridian-VirClia-Diasorin, Meridian-VirClia-SD Biosensor and finally, Meridian-Diasorin-SD Biosensor.

Correlation analysis VIRCLIA - LIAISON

Out of the 313 samples, 65 were positive and 216 were negative for both Liaison® and VirClia®. Eleven samples showed positive results with Diasorin but negative results with VirClia®, while 5 samples tested negative with Liaison but positive with VirClia®. Sixteen samples were equivocal. Refer to Table 1 for results.

The relative sensitivity of VirClia® vs Liaison® was 86% (65/76) and the relative specificity was 98% (216/221)

The agreement between VirClia® and Liaison® was calculated as 94.6% (281/297).

VIRCLIA vs LIAISON	No.
True positive	65
False positive	5
True negative	216
False negative	11
Equivocal	16
Total	313
RELATIVE SENSITIVITY	86%
RELATIVE SPECIFICITY	98%
Agreement	94.6%

Table 1: VirClia® vs. Liaison® results for HP Ag samples

The equivocal results have not been considered for calculation.

Correlation analysis VIRCLIA – SD BIOSENSOR

Out of the 313 samples, 75 were positive and 217 were negative for both SD BIOSENSOR and VirClia®. Nine samples showed positive results with SD BIOSENSOR but negative results with VirClia®, while 5 samples tested negative with SD BIOSENSOR but positive with VirClia®. Seven samples were equivocal. Refer to Table 2 for results.

The relative sensitivity of VirClia® vs SD Biosensor was 89% (75/84) and the relative specificity was 98% (217/222)

The agreement between VirClia® and SD Biosensor was calculated as 95.4% (292/306).

VIRCLIA vs SD BIOSENSOR	No.
True positive	75
False positive	5
True negative	217
False negative	9
Equivocal	7
Total	313
RELATIVE SENSITIVITY	89%
RELATIVE SPECIFICITY	98%
Agreement	95.4%

Table 2: VirClia® vs. SD Biosensor for HP Ag samples

The equivocal results have not been considered for calculation.

Performance analysis VIRCLIA versus consensus result Liaison – SD Biosensor – Meridian

Out of the 313 samples, 68 were positive and 223 were negative for both VirClia® and consensus result. Seven samples showed positive results with Consensus but negative results with VirClia®, while 5 samples tested negative with Consensus result but positive with VirClia®. Ten samples were equivocal. Refer to Table 3 for results.

VIRCLIA vs. CONSENSUS	<i>No.</i>
True positive	68
False positive	5
True negative	223
False negative	7
Equivocal	10
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Total	313
SENSITIVITY	91%
SPECIFICITY	98%
PPV	93.15%
NPV	96.96%
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Agreement	96.0%
Cohen's kappa coefficient (K)	0.906

Table 3: VirClia® vs. Consensus result for HP Ag samples

The Sensitivity of VirClia® vs Consensus was 91% (68/75) and the Specificity was 98% (223/228). The diagnostic performance of the VirClia® assay through a ROC analysis reported a 0.970 area under the curve. See Figure 1.

The agreement between VirClia® and Consensus was calculated as 96.0% (291/303).

The degree of agreement between VirClia® and Consensus was also analysed by Cohen's kappa coefficient (k), showing a value of 0.906; (95% confidence Interval [CI] = 0.854 to 0.958), that is, a "Almost perfect agreement" between the two methods.

The equivocal results have not been considered for calculation.

Performance analysis LIAISON versus consensus result VirClia – SD Biosensor – Meridian

Out of the 313 samples, 66 were positive and 214 were negative for both Liaison® and consensus result. Nine samples showed positive results with Consensus but negative results with VirClia®, while 13 samples tested negative with Consensus result but positive with Liaison®. Eleven samples were equivocal. Refer to Table 4 for results.

LIAISON vs. CONSENSUS	<i>No.</i>
True positive	66
False positive	13
True negative	214
False negative	9
Equivocal	11
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Total	313
SENSITIVITY	88%
SPECIFICITY	94%
PPV	83.54%
NPV	95.96%
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Agreement	92.7%
Cohen's kappa coefficient (K)	0.833

Table 4: Liaison® vs. Consensus result for HP Ag samples

The Sensitivity of Liaison® vs Consensus was 88% (66/75) and the Specificity was 94% (214/227). The diagnostic performance of the Liaison® assay through a ROC analysis reported a 0.922 area under the curve. See Figure 2.

The agreement between Liaison® and consensus was calculated as 92.7% (280/302).

The degree of agreement between Liaison® and Consensus was also analysed by Cohen's kappa coefficient (k), showing a value of 0.833 (95% confidence Interval [CI] = 0.766 to 0.900), that is, a "Almost perfect agreement" between the two methods.

The equivocal results have not been considered for calculation.

Performance analysis SD Biosensor versus consensus result VirClia – Liaison – Meridian

Out of the 313 samples, 69 were positive and 221 were negative for both SD Biosensor and the consensus result. Five samples showed positive results with Consensus but negative results with VirClia®, while nine samples tested negative with Consensus result but positive with SD Biosensor. Nine samples were equivocal. Refer to Table 5 for results.

SD Biosensor vs. CONSENSUS	No.
True positive	69
False positive	9
True negative	221
False negative	5
Equivocal	9
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Total	313
SENSITIVITY	93%
SPECIFICITY	96%
PPV	88.46%
NPV	97.79%
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Agreement	95.4%
Cohen's kappa coefficient (K)	0.891

Table 5: SD Biosensor vs. Consensus result for HP Ag samples

The Sensitivity of SD Biosensor vs Consensus was 93% (69/74) and the Specificity was 96 % (221/230). The diagnostic performance of the SD Biosensor® assay through a ROC analysis reported a 0.961 area under the curve. See Figure 3.

	VirClia®	Liaison®	SD Biosensor
Sensitivity %	91	88	93
Specificity %	98	94	96
Agreement %	96.0	92.7	95.4
Cohen's kappa coefficient (k)	0.906	0.833	0.891
ROC Area Under the Curve	0.970	0.922	0.961

Table 6: Performance results VirClia®, Liaison® and SD Biosensor vs Consensus. Sensitivity, Specificity, Percentage agreement, Kappa coefficient and Area under de Curve

The percentage agreement between SD Biosensor and Consensus was calculated as 95.4% (290/304).

The degree of agreement between SD Biosensor and Consensus was also analysed by Cohen's kappa coefficient (k), showing a value of 0.891 (95%

confidence Interval [CI] = 0.835 to 0.947), that is, a "Almost perfect agreement" between the two methods.

The equivocal results have not been considered for calculation.

Discussion

In this internal retrospective study Vircell has evaluated the HELICOBACTER PYLORI Ag VIRCLIA® MONOTEST and the *H Pylori Ag FIA* assay from SD Biosensor with a panel of samples that had been previously characterized with the Liaison® Meridian *H. pylori SA* from Diasorin.

Apart from assessing the strict correlation between each of the three manufacturers, Vircell aimed to compare the results obtained from VirClia®, SD Biosensor and Liaison assays in relation to consensus result. The Premier Platinum HpSA PLUS ELISA assay from Meridian has been used to test the discrepant samples found among the three different assays being compared: VirClia®, Liaison® and SD Biosensor. The intention was to build a consensus result based on samples reporting positive and negative results from Meridian and two different manufacturers simultaneously, in which the evaluated immunoassay result was excluded to avoid an outperforming bias.

VirClia®

The HELICOBACTER PYLORI Ag VIRCLIA® MONOTEST showed a good agreement (94.6%) with the Liaison®

Meridian *H. pylori SA* when the Diasorin assay is used a reference. The relative Sensitivity and the relative Specificity of the VirClia® assay vs. Liaison® was 86% and 98% respectively.

When The HELICOBACTER PYLORI Ag VIRCLIA® MONOTEST was compared to a Consensus result based on the Liaison®, SD Biosensor and Meridian assays, the Sensitivity, Specificity, and agreement increased to 91%, 98% and 96.0% respectively.

Liaison®

The Liaison® Meridian *H. pylori* SA was compared to a Consensus result based on the VirClia®, SD Biosensor and Meridian assays. The reported Sensitivity, Specificity and agreement was 88%, 94% and 92.7% respectively.

SD Biosensor

The *H Pylori* Ag FIA from SD Biosensor was compared to a Consensus result based on the Liaison®, VirClia® and Meridian assays. The reported Sensitivity, Specificity and agreement was 93%, 96% and 95.4% respectively.

An objective performance of an immunoassay can be evaluated when compared with a consensus result, especially if this consensus is based on the most relevant manufacturers for *Helicobacter pylori* antigen diagnosis: The Meridian ELISA, the particle immunochemiluminescent based assay from Liaison® Diasorin, whose biomaterials also come from Meridian, and the SD Biosensor a lateral Flow assay with fluorescence reading.

When compared results of all three assays versus a consensus result, the reported sensitivity by SD Biosensor and VirClia® was very similar, 93% and 91% respectively, while the Liaison assays seemed to be slightly less sensitive, with an 88% sensitivity.

As for Specificity, the VirClia® assay showed the highest value with a 98% followed by SD Biosensor with a 96% and Liaison with 94%.

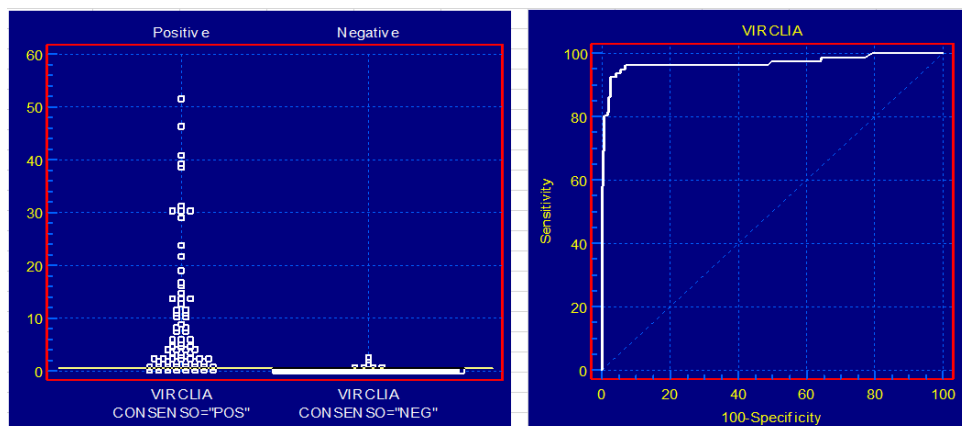


Fig1: ROC Curve. VirClia® assay using Consensus results as a reference. ROC curve sample size of positive group $n = 81$ and sample size of negative group $n = 229$. Area under the ROC curve was 0.970 (95% CI = 0.944 to 0.986)

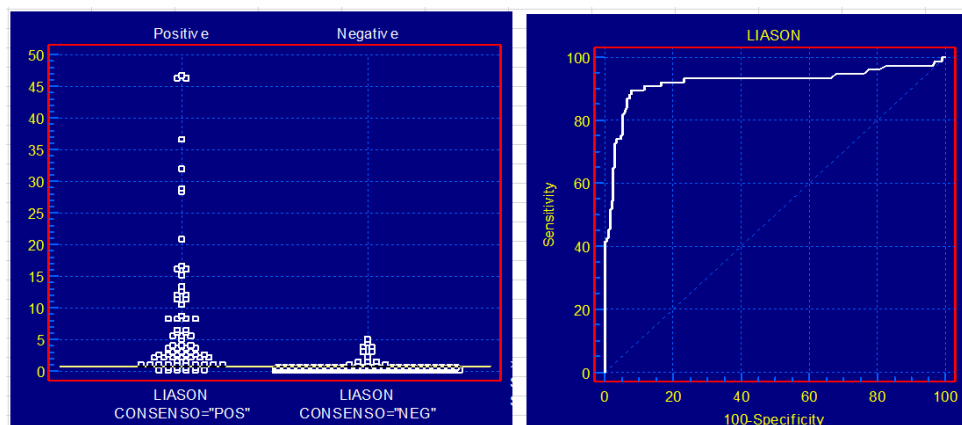


Fig2: ROC Curve. Liaison® assay using Consensus results as a reference. ROC curve sample size of positive group $n = 77$ and sample size of negative group $n = 231$. Area under the ROC curve was 0.922 (95% CI = 0.886 to 0.949)

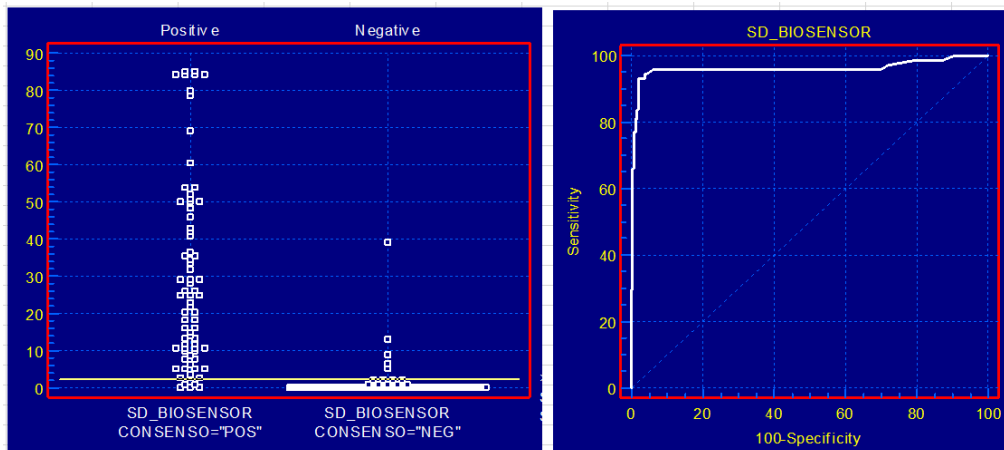


Fig3: ROC Curve. SD Biosensor® assay using Consensus results as a reference. ROC curve sample size of positive group $n = 74$ and sample size of negative group $n = 230$. Area under the ROC curve was 0.961 (95% CI = 0.933 to 0.980)

By using Cohen's kappa, researchers and analysts can get a more comprehensive understanding of the agreement between two methods beyond a simple percentage agreement. The utility of Cohen's kappa in this context lies in providing a measure of agreement that takes into account the possibility of agreement occurring by chance. It corrects for the agreement that would be expected to occur by random chance alone.

In this regard all three evaluated methods showed an excellent kappa coefficient with the consensus result, highest than 0.81 that is considered as “Almost perfect agreement.” Worthwhile to highlight that the VirClia® assay reported the highest coefficient $k=0.906$, followed by SD Biosensor, $k=0.891$ and Liaison® with $k=0.833$

The Diagnostic performance of an immunoassay could be assessed by means of a ROC analysis in which the reported Area Under the Curve represents the best balance between the diagnostic sensitivity and the diagnostic specificity. When the diagnostic performance of the three assays versus consensus was determined by means of a ROC analysis, the VirClia® assay also reported the highest area under the curve (AUC) 0.970, (Figure 1), followed by SD Biosensor with a 0.961 (Figure 2) and Liaison® with 0.922 (Figure 3).

The VirClia® assay demonstrated the strongest agreement in both percentage agreement and Cohen's kappa coefficient, the highest specificity and highest area under the curve when compared to the consensus result derived from all three assessed assays. SD

Biosensor exhibited the highest sensitivity, whereas Liaison® displayed the lower agreement, sensitivity, and specificity in comparison to the consensus result across all three assays as reported in Table 6. According to these results, all three assays VirClia®, Liaison® and SD Biosensor showed a good diagnostic performance for *Helicobacter pylori* antigen detection.

Conclusion

The findings of this study by Vircell suggest that the performance of VirClia®, Liaison®, and SD Biosensor is comparable, while acknowledging inherent differences that may arise among various in-vitro diagnostic manufacturers.

Upon individual assessment of each assay against a consensus result, it is evident that all assays exhibit strong correlation. Specifically, the VirClia® assay demonstrated the highest agreement at 96%, followed by SD Biosensor at 95%, and Liaison at 92%.

Furthermore, SD Biosensor exhibited slightly higher sensitivity, whereas the VirClia® assay demonstrated the slightly higher specificity and slightly higher diagnostic performance in terms of AUC when compared to the consensus result.

Consequently, all VirClia®, Liaison® and SD Biosensor assays demonstrate suitability for routine use in clinical laboratories, particularly in the diagnosis *Helicobacter pylori* antigen in stool samples.

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