# Comparison between Basic Performances of HISCL™ Hepatitis B Core Antibody Reagent and Other Reagents

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*Hepatitis B virus (HBV) reactivation has emerged as a major health concern. It is associated with the use of potent immunosuppressants, including rituximab, in patients with resolved HBV infection who are negative for the hepatitis B surface antigen (HBsAg). The importance of using a hepatitis B core antibody (anti-HBc) kit as a screening tool for such patients has been re-evaluated. We performed a fundamental evaluation of the Sysmex HISCL Anti-HBc assay reagent and compared it with other chemiluminescent reagents using a chemiluminescent enzyme immunoassay. The results indicated that the overall performance of the HISCL Anti-HBc assay reagent was satisfactory. However, each of the study reagents demonstrated both false-negative and false-positive results at times, suggesting that qualitative analysis of near-cutoff or low-titer samples can be a challenge regardless of the reagent used. Although the anti-HBc test is a useful screening tool for patients who have resolved HBV infection and are negative for HBsAg, performing it alone may provide limited accuracy in patients with reduced antibody titers.*

## INTRODUCTION

Practice standards which may prevent patients from hepatitis B virus (HBV) reactivation are essential. The "Guidelines for the Prevention of Hepatitis B Induced by Immunosuppressive Therapy and/or Chemotherapy (revised version)" have been issued by the Ministry of Health, Labour and Welfare study groups on intractable hepatobiliary diseases and standardization of the treatment of viral hepatic diseases, including liver cirrhosis. [2)] The guidelines recommend that patients negative for HBsAg should be tested for hepatitis B core antibody (anti-HBc) and HBs antibody (anti-HBs), and the positive samples should be subjected to HBV-DNA quantification. The guidelines also recommend that HBsAg, anti-HBc, and anti-HBs should be measured using an assay with high sensitivity. Among currently available immunoassays, those involving luminescence are highly sensitive and expected to provide high detection sensitivity and specificity. In this study, we evaluated the basic performance of the HISCL Anti-HBc reagent and compared it with other luminescent assay reagents in terms of differences in qualitative determination, focusing on samples with borderline positive and negative values.

## METHODS

### 1) Materials

The within-run reproducibility was assessed using diluted sample solutions and low-level positive and high-level negative quality control samples. For the assessment of the between-run reproducibility, quality control samples and diluted sample solutions were used. These samples included the following: negative samples (with approximately 0.3 cutoff index [C.O.I.]), near-cutoff samples (with approximately 1.3 C.O.I.), and positive samples (with approximately 7 C.O.I.). The on-board stability was measured using positive samples at three different concentrations.

In this evaluation, 77 samples with borderline values were measured using the anti-HBc test. The results revealed a measurement close to the reference value of 1.0 C.O.I. (C.O.I. ranged between 0.1 and 2.0) by the HISCL Anti-HBc reagent. Each sample was stored at -70°C until measurement. All samples were used after the patients provided consent for this study under the hospital's ethical code.

## 2) Devices and Reagents

We used the chemiluminescent enzyme immunoassay (CLEIA)-based HISCL analyzer with the HISCL Anti-HBc kit (Sysmex Corporation), CLEIA-based Lumipulse Presto II analyzer (hereafter referred to as "Presto") with its Lumipulse Presto Anti-HBc-N reagent (Fujirebio Inc.), and chemiluminescence immunoassay (CLIA)-based Architect Analyzer assay device (hereafter referred to as "Architect") with its Architect HBc II reagent (Abbott Japan Co. Ltd.). The measurement principle is characterized by a double-antigen two-step sandwich assay for HISCL and an antigen-anti-human (IgG) antibody two-step sandwich assay for Presto and Architect (***Fig. 1***).

## 3) Parameters evaluated

*(1) Within-run reproducibility*

The within-run reproducibility (n = 20) was evaluated using diluted sample solution (as a negative sample) and the manufacturer's control sera at two different concentrations (as a positive sample).

*(2) Between-run reproducibility*

Samples showing approximately 0.3, 1.3, and 7.3 C.O.I. were prepared and measured twice daily for 20 days (n = 20) to evaluate the between-run reproducibility.

*(3) On-board stability*

Using a calibration curve measured on day 0, the reagent was stored at 18 ± 1°C. Using anti-HBc positive control sera at three different concentrations, measurement was made every 7 days until day 42.

*(4) Agreement rate*

The anti-HBc level was measured in 77 clinical samples using the HISCL, Presto, and Architect analyzers. The agreement rates between the HISCL and Architect, the HISCL and Presto, and the Presto and Architect were determined.

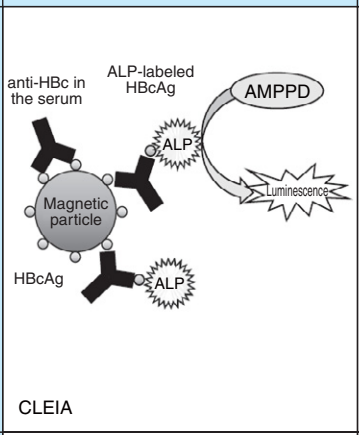
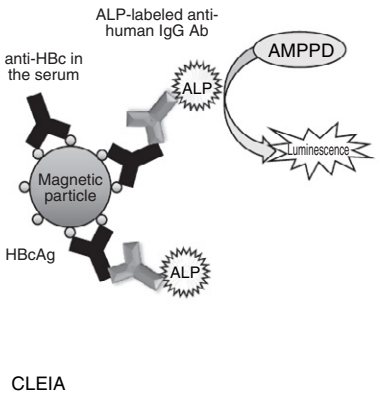
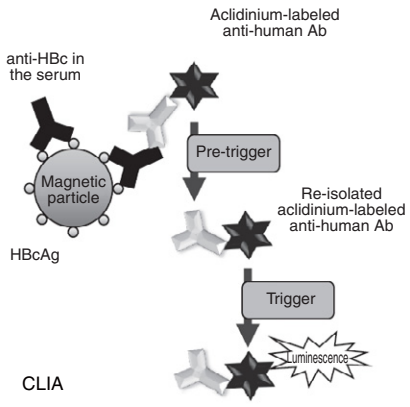
| Reagent name | HISCL™ Anti-HBc Assay kit | Lumipulse Presto® Anti-HBc-N | Architect® HBc II |
|---|---|---|---|
| Reaction principle |  |  |  |
| Reaction flow | Double-HBcAg two-step sandwich | HBcAg-anti-human IgG Ab two-step sandwich | HBcAg-anti-human Ab two-step sandwich |
| Capturing antigen | HBcAg binding to a magnetic particle | HBcAg binding to a magnetic particle | HBcAg binding to a magnetic particle |
| Labeled Ag (Ab) | ALP-labeled HBcAg | ALP-labeled anti-human IgG Ab | Aclidinium-labeled anti-human Ab |

***Fig. 1*** *Measurement principle of each anti-HBc reagent*

*(5) Additional testing on divergent samples*
For samples with divergent measurements between any two reagents, the hepatitis marker levels (HBsAg, anti-HBs, and ALT) were measured. For positive divergent samples measured by the HISCL, an absorption test was also performed using the HBcAg solution.

## RESULTS

*(1) Within-run reproducibility*
Measurement results are shown in ***Table 1***. The diluted sample solution showed a C.O.I. of 0.0 after all twenty measurements, indicating the stability with low-level samples. The positive quality control sample showed a favorable CV ranging from 2.82% to 3.76%.

*(2) Between-run reproducibility*
Using the mean of two measurement results (i.e., in the morning and afternoon) on each measurement day, data on three different samples were collected (n = 40).
The CV ranged from 3.81% to 6.61%, indicating a good reproducibility across the negative sample concentration range.

*(3) On-board stability*
Of all the measurements obtained from the three different positive samples until day 42, the value for sample 2 on day 7 (5.7%) showed the greatest divergence from that on day 0. Overall, the stability was favorable. None of the samples showed a certain increasing or decreasing trend, indicating good stability.

***Table 1*** *Within-run reproducibility for anti-HBc (C.O.I.)*

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| 1 | 0.00 | 4.90 | 9.60 |
| 2 | 0.00 | 4.60 | 9.70 |
| 3 | 0.00 | 4.70 | 10.10 |
| 4 | 0.00 | 4.50 | 9.90 |
| 5 | 0.00 | 4.90 | 9.80 |
| 6 | 0.00 | 4.60 | 9.80 |
| 7 | 0.00 | 4.90 | 9.80 |
| 8 | 0.00 | 4.50 | 9.60 |
| 9 | 0.00 | 4.60 | 10.00 |
| 10 | 0.00 | 4.60 | 9.50 |
| 11 | 0.00 | 5.00 | 9.90 |
| 12 | 0.00 | 4.80 | 9.30 |
| 13 | 0.00 | 5.00 | 9.60 |
| 14 | 0.00 | 5.10 | 9.20 |
| 15 | 0.00 | 4.80 | 9.30 |
| 16 | 0.00 | 4.90 | 9.50 |
| 17 | 0.00 | 4.60 | 9.10 |
| 18 | 0.00 | 4.80 | 9.60 |
| 19 | 0.00 | 4.80 | 9.80 |
| 20 | 0.00 | 4.60 | 9.90 |
| max | 0.00 | 5.10 | 10.10 |
| min | 0.00 | 4.50 | 9.10 |
| Range | 0.00 | 0.60 | 1.00 |
| mean | 0.000 | 4.760 | 9.650 |
| SD | 0.00 | 0.18 | 0.27 |
| C.V.% | - | 3.76 | 2.82 |

***Table 2*** *Between-run reproducibility for anti-HBc The mean of two measurements each (C.O.I.)*

| | | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|---|
| 1 | AM | 0.30 | 1.25 | 7.25 |
| | PM | 0.30 | 1.25 | 7.10 |
| 2 | AM | 0.25 | 1.45 | 6.95 |
| | PM | 0.25 | 1.30 | 7.40 |
| 3 | AM | 0.30 | 1.40 | 7.75 |
| | PM | 0.25 | 1.15 | 7.25 |
| 4 | AM | 0.30 | 1.30 | 7.85 |
| | PM | 0.30 | 1.25 | 7.60 |
| 5 | AM | 0.30 | 1.25 | 7.25 |
| | PM | 0.30 | 1.30 | 7.55 |
| 6 | AM | 0.30 | 1.35 | 7.45 |
| | PM | 0.25 | 1.15 | 7.20 |
| 7 | AM | 0.30 | 1.25 | 7.05 |
| | PM | 0.30 | 1.20 | 7.15 |
| 8 | AM | 0.25 | 1.15 | 6.80 |
| | PM | 0.30 | 1.20 | 7.65 |
| 9 | AM | 0.30 | 1.25 | 7.45 |
| | PM | 0.30 | 1.30 | 7.00 |
| 10 | AM | 0.30 | 1.35 | 6.75 |
| | PM | 0.30 | 1.25 | 7.35 |
| 11 | AM | 0.30 | 1.35 | 7.60 |
| | PM | 0.30 | 1.25 | 7.35 |
| 12 | AM | 0.30 | 1.20 | 7.30 |
| | PM | 0.30 | 1.25 | 7.45 |
| 13 | AM | 0.30 | 1.35 | 6.90 |
| | PM | 0.25 | 1.35 | 7.20 |
| 14 | AM | 0.30 | 1.25 | 6.80 |
| | PM | 0.30 | 1.35 | 7.05 |
| 15 | AM | 0.30 | 1.30 | 6.80 |
| | PM | 0.30 | 1.30 | 7.45 |
| 16 | AM | 0.30 | 1.20 | 7.05 |
| | PM | 0.30 | 1.30 | 6.80 |
| 17 | AM | 0.30 | 1.30 | 7.30 |
| | PM | 0.30 | 1.25 | 6.95 |
| 18 | AM | 0.30 | 1.25 | 7.25 |
| | PM | 0.25 | 1.35 | 7.15 |
| 19 | AM | 0.30 | 1.25 | 7.15 |
| | PM | 0.30 | 1.30 | 7.40 |
| 20 | AM | 0.30 | 1.30 | 7.10 |
| | PM | 0.30 | 1.30 | 7.35 |
| max | | 0.30 | 1.45 | 7.85 |
| min | | 0.25 | 1.15 | 6.75 |
| Range | | 0.05 | 0.30 | 1.10 |
| mean | | 0.291 | 1.278 | 7.230 |
| SD | | 0.02 | 0.07 | 0.28 |
| C.V.% | | 6.61 | 5.16 | 3.81 |

***Table 3*** *Anti-HBc On-board stability for anti-HBc*

| Days | Sample 1 | | Sample 2 | | Sample 3 | |
|---|---|---|---|---|---|---|
| | C.O.I. | vs. day 1 (%) | C.O.I. | vs. day 1 (%) | C.O.I. | vs. day 1 (%) |
| 0 | 3.1 | 100.0 | 19.1 | 100.0 | 35.2 | 100.0 |
| 7 | 3.3 | 105.4 | 20.2 | 105.7 | 36.1 | 102.7 |
| 14 | 3.0 | 96.8 | 19.5 | 101.9 | 35.4 | 100.7 |
| 21 | 3.1 | 101.1 | 18.9 | 98.8 | 35.7 | 101.6 |
| 28 | 3.0 | 96.8 | 19.1 | 99.8 | 35.0 | 99.5 |
| 35 | 3.0 | 95.7 | 18.5 | 96.7 | 36.2 | 103.0 |
| 42 | 3.0 | 97.8 | 19.2 | 100.5 | 34.8 | 99.1 |

*(4) Agreement rate*

The results for 77 clinical samples obtained by the three anti-HBc assays were as follows: 27, 26, and 29 samples tested positive by the HISCL, Presto, and Architect, respectively. In the comparison of the HISCL and Architect, 41 samples tested negative, with 7 and 9 samples testing positive only by HISCL and by Architect, respectively, showing an agreement rate of 61/77 (79.2%) (***Table 4***). In the comparison of the HISCL and Presto, 43 samples tested negative, with 8 and 7 samples testing positive only by HISCL and by Presto, respectively, showing an agreement rate of 62/77 (80.5%) (***Table 5***). In the comparison of the Presto and Architect, 48 samples tested negative, with 3 samples testing positive only by Architect, showing a high agreement rate of 74/77 (96.1%) (***Table 6***).

***Table 4*** *Agreement rate between HISCL and Architect anti-HBc assays*

| anti-HBc test result | | HISCL | | Total |
|---|---|---|---|---|
| | | + | - | |
| Architect | + | 20 | 9 | 29 |
| | - | 7 | 41 | 48 |
| Total | | 27 | 50 | 77 |

Agreement rate: 61/77 = 79.2%

***Table 5*** *Agreement rate between Presto and Architect anti-HBc assays*

| anti-HBc test result | | HISCL | | Total |
|---|---|---|---|---|
| | | + | - | |
| Presto | + | 19 | 7 | 26 |
| | - | 8 | 43 | 51 |
| Total | | 27 | 50 | 77 |

Agreement rate: 62/77 = 80.5%

***Table 6*** *Methods of determination for analytes on BX-3010*

| anti-HBc test result | | Presto | | Total |
|---|---|---|---|---|
| | | + | - | |
| Architect | + | 26 | 3 | 29 |
| | - | 0 | 48 | 48 |
| Total | | 26 | 51 | 77 |

Agreement rate: 74/77 = 96.1%

*(5) Additional testing on divergent samples*

Among the samples, 17 showed divergent test results between the reagents. Among the 17 samples, 9 divergent samples tested negative by HISCL, which also tested negative for HBsAg (***Table 7***). Of these 9 samples, 6 tested positive for anti-HBs, 3 of which (No. 6, 7, and 8) showed a high value of 100.0 mIU/mL or higher. On the contrary, 8 divergent samples tested positive by HISCL, among them, 1 (No. 1) tested positive for HBsAg and HBV-DNA (***Table 8***). An absorption test using HBcAg solution was performed on the above 8 divergent samples testing positive by HISCL. The absorption of anti-HBc was observed in all the samples except for 1 sample (No. 6).

***Table 7*** *Additional testing on negative divergent samples by HISCL*

| No. | anti-HBc (C.O.I) | | | Other makers | | |
|---|---|---|---|---|---|---|
| | HISCL | Presto | Architect | HBsAg (IU/mL) | anti-HBs (mIU/mL) | ALT (U/L) |
| 1 | 0.6 (-) | 0.8 (-) | 1.00 (+) | 0.00 (-) | 23.9 (+) | 48 |
| 2 | 0.4 (-) | 0.8 (-) | 1.06 (+) | 0.01 (-) | 0.5 (-) | 11 |
| 3 | 0.3 (-) | 1.4 (+) | 1.16 (+) | 0.00 (-) | 16.1 (+) | 13 |
| 4 | 0.9 (-) | 1.1 (+) | 1.50 (+) | 0.00 (-) | 0.2 (-) | 37 |
| 5 | 0.4 (-) | 1.1 (+) | 1.60 (+) | 0.00 (-) | 11.7 (+) | 33 |
| 6 | 0.5 (-) | 2.4 (+) | 2.19 (+) | 0.00 (-) | 289.9 (+) | 25 |
| 7 | 0.4 (-) | 2.7 (+) | 2.98 (+) | 0.01 (-) | 121.2 (+) | 20 |
| 8 | 0.4 (-) | 3.0 (+) | 3.34 (+) | 0.00 (-) | 496.6 (+) | 15 |
| 9 | 0.6 (-) | 4.3 (+) | 3.78 (+) | 0.00 (-) | 0.1 (+) | 9 |

***Table 8*** *Additional testing on positive divergent samples by HISCL*

| No. | anti-HBc (C.O.I) | | | Other markers and absorption test | | | | |
|---|---|---|---|---|---|---|---|---|
| | HISCL | Presto | Architect | HBsAg (IU/mL) | anti-HBs (mIU/mL) | ALT (U/L) | HISCL Anti-HBc absorption test (C.O.I.) | Note |
| 1 | 3.7 (+) | 0.2 (-) | 0.71 (-) | 0.06 (+) | 0.0 (-) | 13 | 0.2 | HBV-DNA (+) |
| 2 | 1.1 (+) | 0.2 (-) | 0.45 (-) | 0.00 (-) | 2.2 (-) | 31 | 0.1 | HBsAg negative with resolved HBV infection |
| 3 | 3.0 (+) | 0.7 (-) | 1.15 (+) | 0.00 (-) | 44.8 (+) | 13 | 0.3 | - |
| 4 | 2.2 (+) | 0.1 (-) | 0.16 (-) | 0.00 (-) | 0.0 (-) | 24 | 0.0 | - |
| 5 | 1.1 (+) | 0.1 (-) | 0.23 (-) | 0.00 (-) | 4.3 (-) | 19 | 0.2 | - |
| 6 | 2.9 (+) | 0.1 (-) | 0.39 (-) | 0.01 (-) | 0.0 (-) | 21 | 2.4 | - |
| 7 | 1.9 (+) | 0.2 (-) | 0.39 (-) | 0.00 (-) | 0.0 (-) | n.t. | 0.0 | - |
| 8 | 1.2 (+) | 0.1 (-) | 0.41 (-) | 0.00 (-) | 0.0 (-) | 51 | 0.2 | - |

## DISCUSSION

We evaluated the basic performance of the CLEIA-based HISCL Anti-HBc reagent, and concluded that it is useful for routine testing in terms of its basic performance.
We also focused on the importance of the anti-HBc test as a screening tool for patients negative for HBsAg who have resolved HBV infection, according to the recent guidelines, and compared the near-cutoff (1.0 C.O.I.) performance between the three reagents. It is known that the reactivity of identical immunological assays may differ depending on the difference in reaction flow, the type of labeling material, and the labeled antigens or antibodies. [4] In the anti-HBc test using near-cutoff samples, the test results were reagent dependent. The agreement rate between the Presto and the Architect was as high as 96.1%. However, comparing the HISCL with the Architect, and the HISCL with the Presto, the agreement rate was only about 80% for both the comparisons, suggesting the difference was due to the reaction flow (i.e., the HISCL reagent employs a double-HBcAg two-step sandwich assay, but the other two reagents employ an HBcAg-anti-human (IgG) antibody two-step sandwich assay). The Lumipulse Presto Anti-HBc-N reagent is designed to detect only IgG type anti-HBc, to differentiate chronic hepatitis characterized by increased IgG type anti-HBc from acute hepatitis characterized by increased IgM type anti-HBc. Although the HISCL and the Architect may also have detected IgM type anti-HBc, it was unlikely to be the reason for the disagreement, given the high agreement rate between Presto and Architect. Of all 17 divergent samples, 12 samples were collected for infection testing before surgery or transfusion and 5 samples were collected after resolving HBV infection for the HBsAg screening test to be performed before chemotherapy. The additional testing using these 17 samples revealed that 3 of the 9 samples that tested negative by the HISCL were positive for anti-HBs (without history of vaccination) with a value exceeding 100 mIU/mL. This means that these 3 samples were likely to be derived from patients negative for HBsAg who had resolved HBV infection, as shown by the Architect and Presto. These samples were suspected to be HISCL false-negative results. On the contrary, 8 divergent samples tested positive by the HISCL. Of these, 1 sample tested positive by the HBV-DNA assay, and another sample was confirmed to be collected from a patient negative for HBsAg, who had resolved HBV infection. At least these 2 samples were anti-HBc positive, which were likely to be false-negative results from the Architect and Presto. In particular, the sample testing positive by the HBV-DNA assay was collected before chemotherapy, with a negative anti-HBs test result. It was recently reported that among B-cell non-Hodgkin lymphoma patients with resolved HBV infection who were anti-HBc positive and anti-HBs negative at the start of the treatment, only approximately 20% experienced HBV reactivation. [5] This finding further emphasizes the importance of anti-HBc testing.
The anti-HBc test is effective as a screening tool for patients negative for HBsAg who have resolved HBV infection. However, the accuracy of test results may vary especially if the sample value is near-cutoff. In particular, immunosuppressed patients can test as false-negative for both antibody types. To prevent hepatitis B reactivation, it is crucial to concurrently test for anti-HBc and anti-HBs according to the guidelines.

## CONCLUSION

We evaluated the basic performance of the HISCL Anti-HBc reagent, and compared the performance of three antibody assay reagents (HISCL, Presto, and Architect) using near-cutoff samples. The anti-HBc test is a useful screening tool for patients negative for HBsAg who have resolved HBV infection. However, the evaluation revealed that the test results may vary. Therefore, it is crucial to concurrently test for anti-HBc and anti-HBs according to the guidelines. In some patients, the use of immunosuppressants is also associated with false-negative results for anti-HBc. In these patients an effort should be taken to minimize the risk of hepatitis B reactivation by concurrently testing anti-HBs and HBsAg, and a reagent with high sensitivity should be selected for testing.

***References***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