การวิเคราะห์ค่าก้ำกึ่งและค่าบวกต่ำของผลการทดสอบแอนติเจนส่วนผิวรอบนอกไวรัสตับอักเสบบีด้วยเครื่องทดสอบอัตโนมัติต่างชนิด

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บทคัดย่อ

แอนติเจนส่วนผิวรอบนอกของไวรัสตับอักเสบบี (HBsAg) นิยมใช้บ่งชี้การติดเชื้อไวรัสในกระแสเลือด อย่างไรก็ตาม ผลการทดสอบ HBsAg ที่ได้อาจเป็นผลบวกปลอม การศึกษานี้มีวัตถุประสงค์เพื่อหาจุดตัดที่เหมาะสมในการแปลผลบวกของ HBsAg ที่แท้จริงในกลุ่มที่ได้ค่าก้ำกึ่งและค่าบวกต่ำ โดยเป็นการศึกษาข้อมูลย้อนหลังจากผลตรวจในตัวอย่างเลือดที่ส่งทดสอบ HBsAg ณ ภาควิชาพยาธิวิทยา หน่วยภูมิคุ้มกันวิทยา โรงพยาบาลรามาธิบดี ในช่วงเดือนมกราคม พ.ศ. 2554 ถึง เดือนพฤษภาคม พ.ศ. 2560 โดยวิเคราะห์ข้อมูลจากผลทดสอบที่ตั้งต้นจากเครื่องอัตโนมัติ Architect ที่ให้ค่า signal-to-cutoff ratios (s/co) ตั้งแต่ 0.9 ถึง 20 จำนวน 651 ราย เปรียบเทียบกับผลทดสอบจากเครื่องอัตโนมัติที่สอง ได้แก่ Elecsys, Vitros หรือ Vidas เพื่อยืนยันการแปลผล รวมทั้งศึกษาเปรียบเทียบค่า HBsAg กับปริมาณสารพันธุกรรมไวรัสในเลือด (viral load) ผลการศึกษาพบว่า ผลทดสอบ HBsAg ด้วยเครื่องอัตโนมัติที่สองยืนยันการแปลผลบวก (positive) ร้อยละ 59 แปลผลคลุมเครือ (intermediate) ร้อยละ 18 และแปลผลลบ (negative) ร้อยละ 23 และเมื่อผลการทดสอบจากเครื่อง Architect ให้ค่า s/co ≥10 จะยืนยันการแปลผลบวกถึงร้อยละ 98 และแปลผลคลุมเครือเพียงร้อยละ 2 โดยปราศจากการแปลผลลบ การคำนวณค่าความสัมพันธ์ระหว่าง HBsAg จากเครื่อง Architect กับเครื่องทดสอบอัตโนมัติที่สอง Elecsys, Vitros และ Vidas พบว่าให้ค่าใกล้เคียงกันจากมากไปน้อยตามลำดับดังนี้ r = 0.75, 0.65 และ 0.60 ส่วนการตรวจพบปริมาณสารพันธุกรรมไวรัสในเลือดในกลุ่มประชากรศึกษาครั้งนี้พบเพียงร้อยละ 47 เมื่อพิจารณาจากผลการศึกษาทั้งหมด สรุปได้ว่า ค่าก้ำกึ่งและค่าบวกต่ำของ HBsAg จากเครื่องมือ Architect ที่ให้ค่า s/co <5 ควรตรวจยืนยันด้วยเครื่องอัตโนมัติที่สองก่อนการแปลผล แต่เมื่อค่า s/co ≥10 ขึ้นไปสามารถทำนายผลบวกของ HBsAg ได้อย่างแม่นยำ

คำสำคัญ: เครื่องทดสอบอัตโนมัติสำหรับตรวจหาแอนติเจนส่วนผิวรอบนอกไวรัสตับอักเสบบี ค่าก้ำกึ่งและค่าบวกต่ำของแอนติเจนส่วนผิวรอบนอกไวรัสตับอักเสบบี

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Analysis of Borderline and Low Level Reactive Hepatitis B Virus Surface Antigen (HBsAg) by Different Automated HBsAg Assays

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Abstract

Hepatitis B virus (HBV) surface antigen (HBsAg) has been used as the first-line marker to detect HBV infection. However, HBsAg assay could give false positive result. The objective of this study was to define the signal cutoff in borderline and low level reactive HBsAg results in order to interpret positive HBsAg results correctly. The retrospective study of data collected from automated HBsAg assays at Clinical Immunology Unit, Department of Pathology, Ramathibodi Hospital from January 2011 to May 2017 was conducted. The total 651 HBSAg test results that Architect HBsAg assay showed signal-to-cutoff (s/co) ratios 0.9 to 20 were included in this study. The interpretation of HBsAg results was confirmed by second automated HBsAg assays, either Elecsys HBsAg II, Vitros HBsAg or Vidas HBsAg. In addition, the test results of HBsAg assay were compared with HBV viral load.

The second HBsAg assays confirmed 59% positive HBsAg, 18% intermediate HBsAg, and 23% negative HBsAg. When Architect HBsAg showed signal ≥10 s/co ratios, the second assays confirmed 98% positive HBsAg and 2% intermediate HBsAg without negative HBsAg. The Architect HBsAg assay correlated with the second assays; Elecsys HBsAg II, Vitros HBsAg and Vidas HBsAg, (r = 0.75, 0.65 and 0.60 respectively). However, only 47% of total samples were positive for HBV viral load.

In conclusion, borderline and low level reactive Architect HBsAg results that showed signal <5 s/co ratios needed confirmation by the second assays before HBsAg interpretation and report. Architect HBsAg test results that showed signal ≥10 s/co ratios could be precisely predicted positive HBsAg results.

Keywords: HBsAg, Automated HBsAg assays, Borderline and low level reactive HBsAg

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Introduction

Hepatitis B virus (HBV) infection is a global health problem with more than 200 million people infected.[1, 2] About 5.1 percent of Thai population has been facing chronic HBV infection.[3] First-line test used to indicate HBV infection is HBV surface antigen (HBsAg) test, therefore, HBsAg test is performed as screening test for blood donation, pre-operation, and health checkup. In general, HBsAg positive results will be tested along with other HBV serological profile and HBV viral load is recommended to determine HBV status and treatment.

Reactive HBsAg results should be confirmed by neutralizing assay or second HBsAg assay before HBsAg interpretation.[4-6] The laboratory test algorithm for second HBsAg assay is suggested. When the first assay shows non-reactive result, the interpretation will be "negative" HBsAg. When both assays show reactive result, the interpretation will be "positive" HBsAg. And when both assays show discordant result, the interpretation will be "indeterminate" HBsAg.[7] Indeterminate HBsAg usually needs molecular testing to confirm HBV infection status.[8]

The HBsAg test results that give high cutoff signal by the first assay are usually true positive HBsAg, and the second assay is always concordant. In contrast, the low cutoff signal HBsAg test results by the first assay could be false positive HBsAg and the second assay is necessary for confirmation.[9, 10] This study aimed to determine the cutoff of Architect HBsAg assay (as the first assay) that could differentiate between true and false positive HBsAg to avoid the unnecessary confirmation. In addition, the result of HBsAg assays was further compared with HBV viral load.

Materials znd Methods

HBsAg Assays

Four HBsAg assays namely Architect HBsAg, Elecsys HBsAg II, Vitros HBsAg, and Vidas HBsAg were used for HBsAg detection. Architect HBsAg (HBsAg Qualitative; Abbott Ireland Diagnostics Division, Sligo, Ireland) was a chemiluminescent microparticle immunoassay (CMIA) using anti-HBsAg coated microparticles (mouse, monoclonal, IgM and IgG) and anti-HBsAg acridinium-labeled conjugate (goat, IgG). Elecsys HBsAg II (Roche Diagnostics, Mannheim, Germany) was an electrochemiluminescence immunoassay (ECLIA) using two biotinylated anti-HBsAg (mouse, monoclonal) and two anti-HBsAg ruthenium-labeled conjugate (mouse, monoclonal and sheep, polyclonal). Vitros HBsAg (Ortho-Clinical Diagnostics, Bridgend CF35 5PZ, United Kingdom) was a luminogenic substrates enzyme immunoassay using anti-HBsAg coated wells (mouse, monoclonal) and anti-HBsAg horseradish peroxidase-labeled conjugate (mouse, monoclonal). Vidas HBsAg (bioMérieux S.A, Marcy l'Etoile, France) was an enzyme-linked fluorescent immunoassay (ELFA) using two anti-HBsAg coated solid

phase (mouse, monoclonal) and anti-HBsAg biotin-labeled conjugate (goat, polyclonal).

The test results were derived from the signal of sample divided by the signal of cutoff. The assays had their own reporting units e.g. Architect HBsAg used "S/CO = sample/cutoff", Elecsys HBsAg II used "COI = cutoff index", Vitros HBsAg used "s/c = signal/cutoff", and Vidas HBsAg used "TV = test value". this study simplified the unit into one "signal-to-cutoff (s/co) ratios" for all HBsAg assays. Architect HBsAg, Elecsys HBsAg II, and Vitros HBsAg; defined s/co ratios ⩾1 as reactive result and <1 as non-reactive result. Only Vidas HBsAg (HBL protocol) defined s/co ratios ⩾0.1 as reactive result and <0.1 as non-reactive result.

HBsAg Testing Algorithm

Architect HBsAg was chosen as the first assay, the second assay was either Elecsys HBsAg II, Vitros HBsAg, or Vidas HBsAg. Architect HBsAg results that gave <0.9 s/co ratios were interpreted as negative HBsAg. Architect HBsAg results that gave ⩾0.9 s/co ratios were re-tested by Architect HBsAg assay. If the re-test results gave <1.0 s/co ratios, interpretation would be negative HBsAg. If not, the result would be further confirmed by the second assay. When the second assay showed reactive result, HBsAg interpretation would be positive. If not, HBsAg interpretation would be indeterminate. The test algorithm was adapted from "two-assay serological testing strategy" WHO guidelines on Hepatitis B and C testing 2017.[7] (Fig. 1A).

HBV Viral Load Assay

HBV viral load was performed on Abbott Real Time HBV assay (0.5 mL sample volume protocol; Abbott Molecular Inc., IL 60018, USA) using PCR technology combined with real time fluorescent detection to quantitate HBV DNA. The measurable range was 10^1-10^9 IU/mL. The result was interpreted as "undetectable" if HBV viral load <10 IU/mL, and as "detectable" if HBV viral load ⩾10 IU/mL.

Study Population

The data of HBsAg testing was retrieved from January 2011 to May 2017 at Clinical Immunology Unit, Department of Pathology, Ramathibodi Hospital, Bangkok, Thailand. Total number was 192,086 cases; positive HBsAg 11,921 cases, negative HBsAg 179,959 cases, and indeterminate HBsAg 206 cases.

Borderline HBsAg (required retesting) signal was 0.9 to <1 s/co ratios, and low level reactive HBsAg (required confirmatory testing) signal was 1 to 4 s/co ratios by Elecsys HBsAg assay.[6] In order to cover all cases with borderline and low level reactive HBsAg, total 651 cases that Architect HBsAg (as the first assay) gave signal between 0.9 to 20 s/co ratios were selected for this study. The results of Architect HBsAg were compared with the

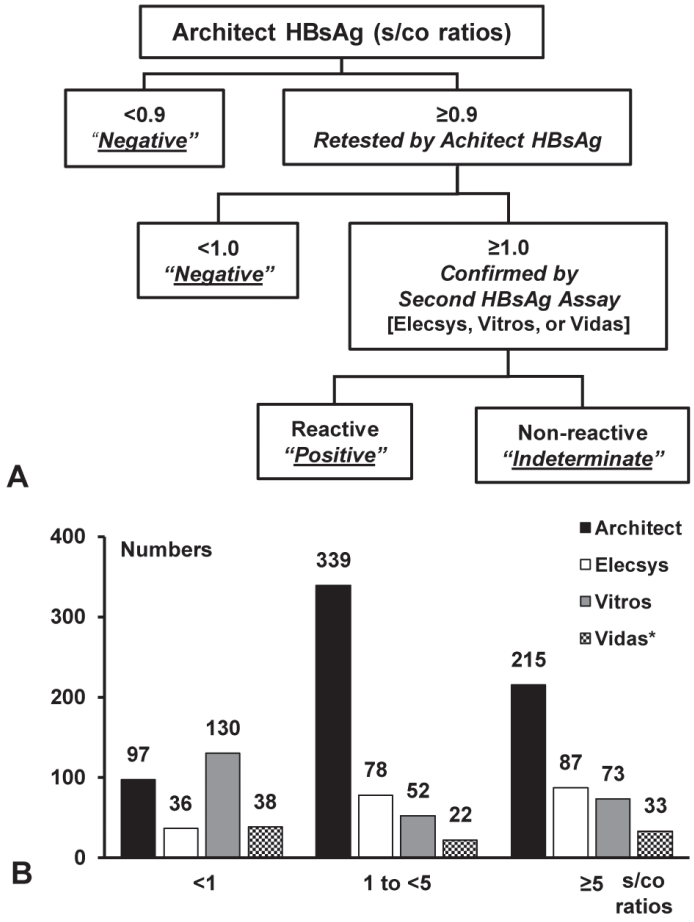

Fig. 1 HBsAg testing algorithm and case distribution. (A) HBsAg testing algorithm and HBsAg interpretation, (B) Distribution of cases based on HBsAg signaling (s/co ratios), *signal of Vidas HBsAg was <0.1, 0.1 to <0.5, and ≥0.5 s/co ratios respectively.

results of second HBsAg assays (Elecsys HBsAg II, Vitros HBsAg, or Vidas HBsAg) and HBV viral load. The study protocol was approved by Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (ID 05-60-76).

Statistical Analysis

The correlation between HBsAg assays and HBV viral load was demonstrated by Pearson correlation coefficient. The diagnostic efficacy of HBsAg Architect with HBsAg interpretation and HBV viral load was demonstrated by ROC curve analysis. The statistical analysis was calculated by using MedCalc Version 12.7.7.

Results

Distribution of cases based on HBsAg signal

In total 651 cases with signal between 0.9 to 20 s/co ratios by Architect HBsAg, most cases showed signal 1 to <5 s/co ratios (339 cases). Vitros HBsAg was largely used as the second assay (255 cases), following by Elecsys HBsAg II and Vidas HBsAg (201 cases, 93 cases). The majority of the cases by Elecsys HBsAg II showed signal ⩾5 s/co ratios (87 cases). The majority by Vitros HBsAg showed signal <1 s/co ratios (130 cases). The cases of Vidas HBsAg equally distributed along the signal range. (Fig. 1B).

Comparison of the second HBsAg assays with Architect HBsAg signaling

Most cases with Architect HBsAg 0.9 to 20 s/co ratios (82%) gave reactive results by Elecsys HBsAg II, but only a half (49% and 59%) by Vitros HBsAg and Vidas HBsAg, as shown in Table 1. The increasing of Architect HBsAg signal was associated with the increasing of percent reactivity by the

Table 1 Percent reactivity by the second HBsAg assays based on s/co ratios of Architect HBsAg

s/co ratios	%Reactivity of HBsAg (No. Reactive / No. Tested) by		
Architect HBsAg	Elecsys	Vitros	Vidas
0.9 to <1	33% (1/3)	0% (0/7)	33% (2/6)
1 to <5	75% (82/110)	33% (57/171)	51% (33/65)
5 to 20	93% (82/88)	88% (68/77)	91% (20/22)
Total	**82% (165/201)**	**49% (125/255)**	**59% (55/93)**

Table 2 Percent HBsAg interpretation based on s/co ratios of Architect HBsAg

s/co ratios	%HBsAg Interpretation (No. Interpreted / Total No.)		
Architect HBsAg	Positive	Negative	Indeterminate
0.9 to <1	0% (0/97)	95% (92/97)	5% (5/97)
1 to <5	52% (178/339)	17% (57/339)	31% (104/339)
5 to 20	95% (205/215)	1% (2/215)	4% (8/215)
10 to 20	98% (102/104)	0% (0/104)	2% (2/104)
Total	**59% (383/651)**	**23% (151/651)**	**18% (117/651)**

second assays (especially Elecsys HBsAg II). Notably, the cases with Architect HBsAg 5 to 20 s/co ratios showed >80% reactivity by any second HBsAg assays; 93%, 88%, and 91% by Elecsys HBsAg II, Vitros HBsAg, and Vidas HBsAg respectively. The cases with Architect HBsAg 1 to 5 s/co ratios showed moderate reactivity by the second assays; 75%, 33%, and 51% by Elecsys HBsAg II, Vitros HBsAg, and Vidas HBsAg, respectively. However, only a small number of cases with Architect HBsAg 0.9 to <1 s/co ratios showed reactive results by the second assays; 33%, 0%, and 33% by Elecsys HBsAg II, Vitros HBsAg and Vidas HBsAg, respectively.

The correlation between Architect HBsAg and the second assays was demonstrated in Fig. 2. Architect HBsAg showed strong correlation with Elecsys HBsAg II (r = 0.75) [95%CI 0.68 to 0.80, p <0.0001], and moderate correlation with Vitros HBsAg (r = 0.60) [95%CI 0.45 to 0.72, p <0.0001] and Vidas HBsAg (r = 0.65) [95%CI 0.58 to 0.72, p <0.0001].

Comparison of HBsAg interpretation with Architect HBsAg signal

The cases with Architect HBsAg signal between 0.9 to 20 s/co ratios gave 59% positive predictive value (PPV) and 23% negative predictive value (NPV) for HBsAg interpretation indicating a poor performance of prediction (Table 2). However, the performance of prediction was varied across the signal range.

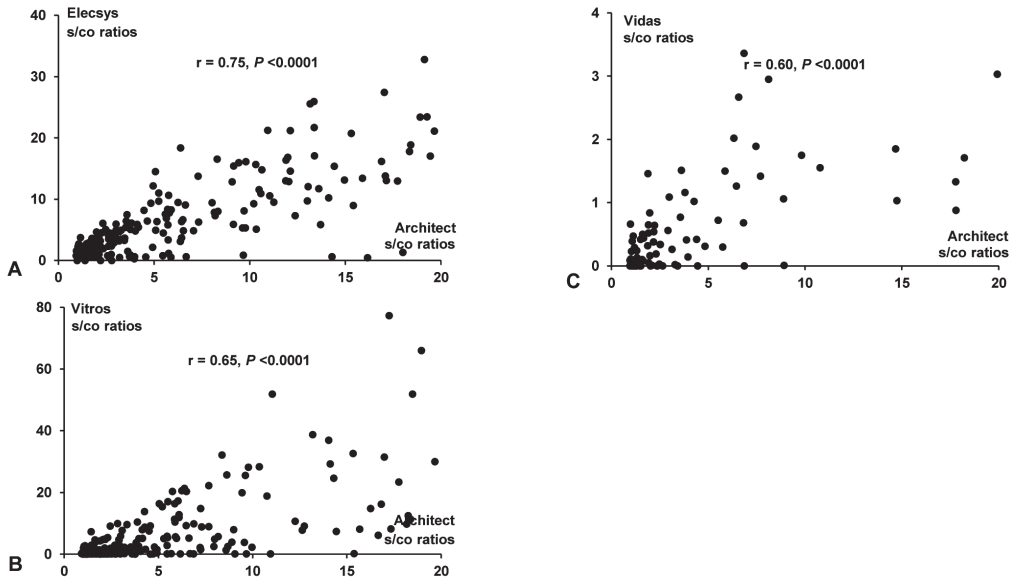

Fig. 2 Correlation between Architect HBsAg with the second HBsAg assays. (A) Architect HBsAg with Elecsys HBsAg II, (B) Architect HBsAg with Vitros HBsAg, and (C) Architect HBsAg with Vidas HBsAg

The cases with Architect HBsAg signal between 0.9 to <1 s/co ratios showed a good performance of negative prediction, 0% PPV and 95% NPV (5% of cases showed non-reactive result by the second assay). The cases with Architect HBsAg 1 to <5 s/co ratios showed a poor performance of positive prediction, 52% PPV and 17% NPV. The cases with Architect HBsAg ⩾5 to 20 s/co ratios showed a good performance of positive prediction, 95% PPV and 1% NPV (1% of cases showed non-reactive result by Architect retesting, and 4% of cases showed non-reactive result by the second assay). In addition, the cases with Architect HBsAg ⩾10 to 20 s/co ratios showed an excellent performance of positive prediction, 98% PPV and 0% NPV (2% of cases showed non-reactive result by the second assay).

Area under receiver operating characteristic curve (AUROC) of Architect HBsAg with positive HBsAg showed an excellent performance at area under the curve (AUC) 0.95. Maximal Youden's index was at the cutoff >1.53 s/co ratios with 91% sensitivity [95%CI 88% to 94%] and 84% specificity [95%CI 77% to 90%], the data of indeterminate HBsAg was excluded before analysis (Fig. 3A).

Comparison of HBsAg assays with HBV viral load

A half of cases by three HBsAg assays showed detectable HBV viral load; 47%, 46%, and 48% by Architect HBsAg, Elecsys HBsAg II, and Vitros HBsAg, respectively. But only a third of cases by Vidas HBsAg (34%) was detectable by viral load assay (Table 3).

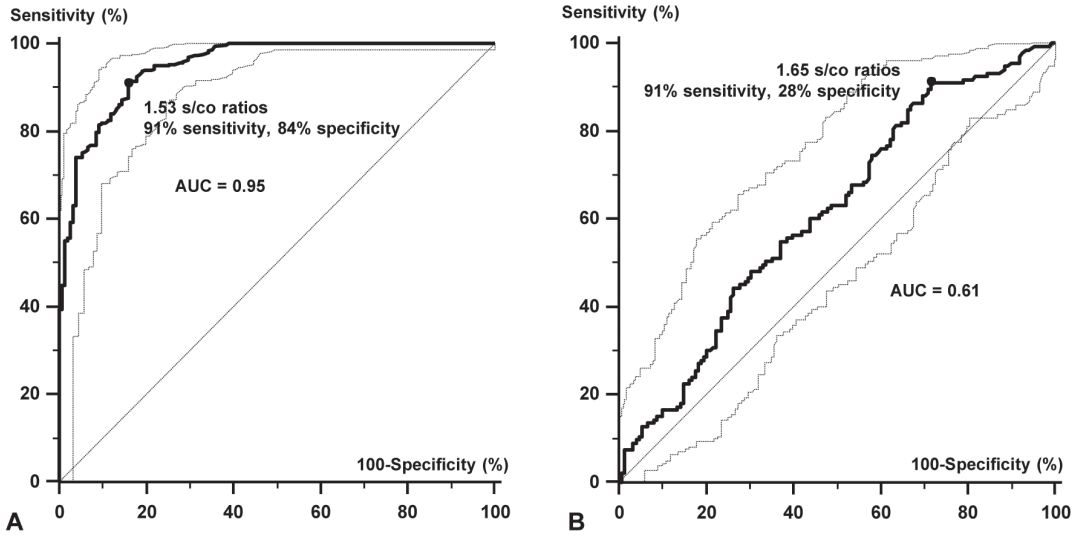

Fig. 3 ROC curve analysis of Architect HBsAg. (A) Architect HBsAg with positive HBsAg, and (B) Architect HBsAg with detectable HBV viral load

Table 3 Percent detectable HBV viral load based on s/co ratios of HBsAg assays

HBsAg (s/co ratios)	%Detectable HBV Viral Load (No. Detectable / No. Tested) by			
	Architect	Elecsys	Vitros	Vidas
<1*	20% (1/5)	20% (1/5)	25% (6/24)	0% (0/6)
1 to <5†	40% (57/141)	38% (18/48)	53% (16/30)	36% (5/14)
≥5‡	56% (75/135)	55% (33/60)	57% (25/44)	44% (8/18)
Total	**47% (133/281)**	**46% (52/113)**	**48% (47/98)**	**34% (13/38)**

*Vidas <0.1, †Vidas 0.1 to <0.5, and ‡Vidas ≥0.5

A quarter of non-reactive cases (<1 s/co ratios) by three HBsAg assays showed detectable HBV by viral load assay; 20%, 20%, and 25% by Architect HBsAg, Elecsys HBsAg II, and Vitros HBsAg, respectively, but none by Vidas HBsAg (<0.1 s/co ratios). And a half of reactive cases (⩾1 or ⩾0.1 s/co ratios) by any HBsAg assays showed detectable HBV by viral load assay.

AUROC of Architect HBsAg with detectable HBV by viral load assay showed a poor performance at AUC 0.61. Maximal Youden's index was at the cutoff >1.65 s/co ratios with 91% sensitivity [95%CI 85% to 95%] and 28% specificity [95%CI 21% to 36%] (Fig. 3B). The correlation between HBsAg assays and HBV viral load was demonstrated in Fig. 4. All HBsAg assays showed a poor correlation with HBV viral load; Architect HBsAg, r = 0.21 [95%CI 0.10 to 0.32, p = 0.0040], Elecsys HBsAg II, r = 0.21 [95%CI 0.02 to 0.38, p = 0.0265], Vitros HBsAg, r = 0.25 [95%CI 0.05 to 0.42, p = 0.0146], and Vidas HBsAg, r = 0.30 [95%CI -0.03 to 0.56, p = 0.0713]. However, the increase HBsAg signal was associated with the increasing of chance to detect HBV by viral load assay.

Comparison of HBsAg interpretation with HBV viral load

A half of cases showed detectable HBV by viral load assay (47%) (Table 4). A quarter of negative HBsAg and indeterminate HBsAg showed detectable HBV by viral load assay (25% and 23%), and a half of positive HBsAg showed detectable HBV viral load (51%). Therefore, HBsAg interpretation with Architect HBsAg 0.9 to 20 s/co ratios was poorly predicted the detectability of HBV viral load.

Discussion

The HBsAg test results in cases with Architect HBsAg <5 s/co ratios give a poor performance to predict the reactivity of second HBsAg assays and positive HBsAg. But the

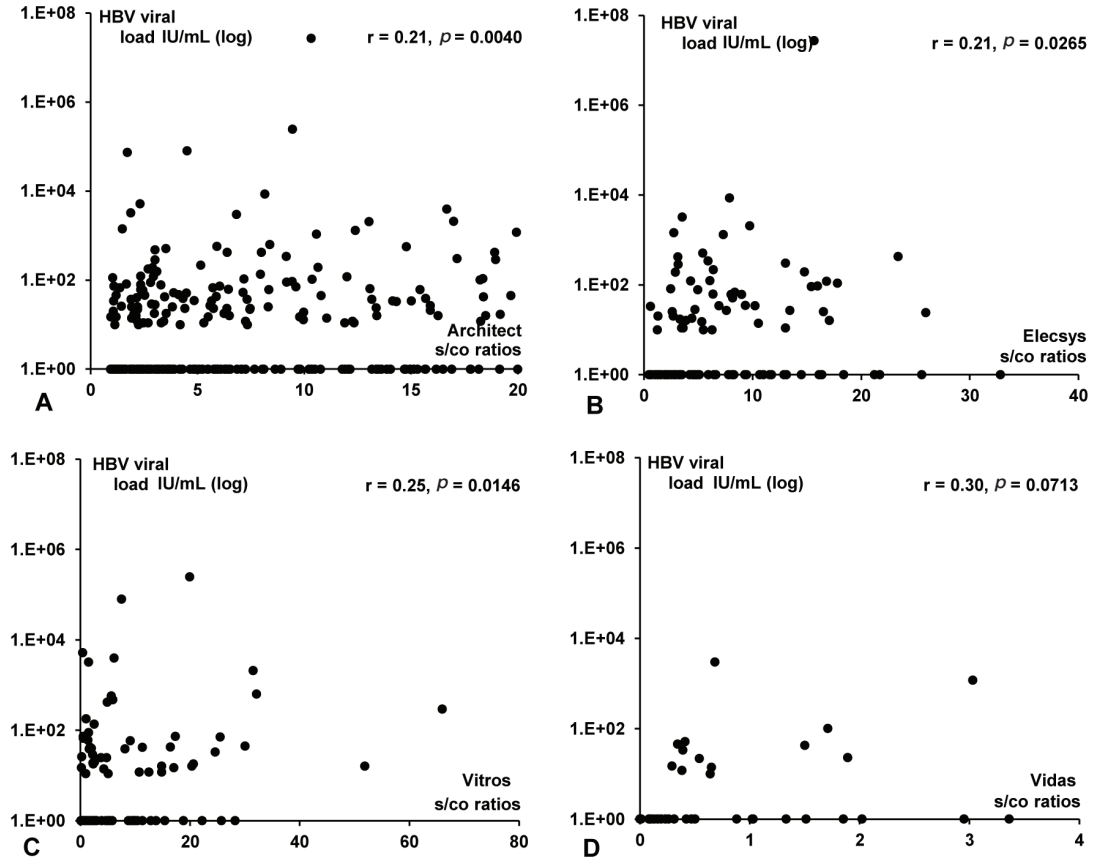

Fig. 4 Correlation between HBsAg assays with HBV viral load. (A) Architect HBsAg, (B) Elecsys HBsAg, (C) Vitros HBsAg, and (D) Vidas HBsAg

Table 4 Percent detectable HBV viral load based on HBsAg interpretation

HBsAg Interpretation	%Detectable HBV Viral Load (No. Detectable / No. Interpreted)
Negative	25% (1/4)
Indeterminate	23% (7/30)
Positive	51% (125/247)
Total	**47% (133/281)**

cases with Architect HBsAg ⩾5 s/co ratios show a good performance of prediction, and the performance is better when the signal ⩾10 s/co ratios. Therefore, Architect HBsAg <5 s/co ratios might be false positive, further confirmation by second HBsAg assays is needed. While Architect HBsAg ⩾10 s/co ratios is true positive HBsAg, further confirmation is then unnecessary. Architect HBsAg shows moderate to strong correlation with the second assays, and the performance to predict positive HBsAg is excellent at AUROC >0.9. Each HBsAg assay shows a distinct performance to detect HBsAg, especially when the signal is low. These are due to different antibodies (IgG or IgM, polyclonal or monoclonal antibodies, certain epitope recognition) that greatly influence the performance of assay are used.[11, 12]

All HBsAg assays show a poor performance to predict detectable HBV viral load, including Architect HBsAg at AUROC <0.7. The correlation between HBsAg assays and HBV viral load is poor, and only a half of cases with positive HBsAg shows detectable viral load. Therefore, reactive HBsAg or positive HBsAg could not automatically indicate the presence of HBV genomes, especially in low signaling. The presence of HBV genomes but absence of HBsAg is called occult HBV infection, occasionally related to mutant HBsAg[13, 14] and the performance to detect mutant HBsAg was varied among the assays.[10, 11, 15] The cases that show discordant results between the assays (indeterminate HBsAg) HBV molecular testings are then required to differentiate between occult HBV infection and false reactive HBsAg. The presence of HBsAg but absence of HBV genomes is caused by several reasons including false reactive HBsAg, inadequate sensitivity of HBV molecular test, inactive carrier, current anti-viral medication and vaccination. Therefore, the HBV status in this population should not be based on the result of HBsAg alone. Additional HBV serology, HBV molecular testing, and clinical information are necessary to determine the HBV status.

The result in this report was concordant with the previous studies.[4-6] The cases with low HBsAg signaling might be false positive HBsAg and the second assay is necessary for confirmation. However, adjusting the cutoff higher in order to reduce the confirmation could save cost and time. The adjusted cutoffs are different depending on the type of assay. One study used cutoff >6 s/co ratios by radioimmunoassay (RIA) and >1 absorbance by enzyme immunoassay (EIA).[4] Other two studies used cutoff >1.785 s/co ratios[5] and >4 s/co ratios[6] by electrochemiluminescence immunoassay (ECLIA). And this study used cutoff >10 s/co ratios by chemiluminescent microparticle immunoassay (CMIA).

This study has some limitation in gaining more insightful information. First, there was bias in HBsAg interpretation because Architect HBsAg was served as the first assay.

When Architect HBsAg showed signal <0.9 s/co ratios, the interpretation was negative HBsAg without further evaluation by other assays. Second, the incomplete data of the second assays (Elecsys HBsAg II, Vitros HBsAg, and Vidas HBsAg) and the selection of the second assays was depended on the laboratory testing algorithm at that time. Third, not all cases with positive HBsAg were tested by HBV viral load assay and lack of clinical data for better analysis.

Conclusion

Architect HBsAg test results that gave 0.9 to 20 s/co ratios show moderate to strong correlation with Vidas HBsAg, Vitros HBsAg, and Elecsys HBsAg II. Architect HBsAg test results show a good performance to predict positive HBsAg when the signal is ⩾5 s/co ratios (95% PPV and 1% NPV) and show an excellent performance when the signal is ⩾10 s/co ratios (98% PPV and 0% NPV). However, all HBsAg assays show a poor performance to predict detectable and the correlation with HBV viral load assay.

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