

Original Article

Confirmation of HBsAg repeatedly reactive in blood donors at Siriraj Hospital

Yuwadee Wanayutthasin¹, Kamol Suwannakarn¹, Iyarat Thaipisuttikul¹ and Parichart Permpikul²

¹Department of Microbiology; ²Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University

Abstract:

Background: Hepatitis B surface antigen is a mandatory screening test for every unit of donated blood. According to the manufacturer's instruction, HBsAg repeatedly reactive (HBsAg RR) needs to be confirmed for a positive result for proper donor management. However, this was not routinely performed in Thailand because of the high prevalence of HBsAg in the past and the extra cost of confirmatory testing. Nowadays, the prevalence of HBV infection has been declined due to the extended program of immunization (EPI program) which started in 1992. **Objective:** To study the essential of confirmation of HBsAg, anti-HBc, and anti-HBs in HBsAg RR blood donors at Siriraj Hospital. **Materials and Methods:** During January 2014 to May 2016, donated blood at Siriraj Hospital was screened for HBsAg by HBsAg Quali II (Architect, Abbott laboratory). Five hundred and twenty five HBsAg RR blood donors were recruited for neutralization by HBsAg Quali II confirmatory test (Architect, Abbott laboratory), and also were tested for anti-HBc and anti-HBs. **Results:** The prevalence of HBsAg RR in this study was 0.50%. From 525 tested samples, 475 samples (90.5%) gave confirmed HBsAg positive results, while only 50 samples (9.5%) were not. The high signal of HBsAg ($s/co > 300$) was correlated with 100% positive predictive value (PPV). In confirmed HBsAg RR group, the anti-HBc and anti-HBs study indicated acute HBV infection in 5 cases (1.05%), chronic HBV infection in 456 cases (96.00%) and surprisingly 14 cases (2.95 %) showed the uncommon coexistence of confirmed HBsAg and anti-HBs. There was no confirmed HBsAg in isolated positive anti-HBs group. **Conclusion:** When performing routine HBsAg screening test in donated blood using HBsAg Quali II (Architect, Abbott Laboratory), we recommend to do the HBsAg confirmatory test especially in low HBsAg signal group ($s/co \leq 300$) in order to prevent falsely permanent defer of blood donors.

Keywords : ● HBsAg neutralization ● Blood donor ● Coexistence HBsAg and anti-HBs

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Correspondence should be addressed to Assoc. Prof. Parichart Permpikul, MD., Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. E-mail: parichart.per@mahidol.ac.th

นิพนธ์ต้นฉบับ

การตรวจยืนยันผลตรวจของเอนติเจนบันผิวไวรัสตับอักเสบบีในผู้บริจากเลือดของโรงพยาบาลศิริราช

ยุวดี วนายุทธคิลป์¹ กลุ่ม สุวรรณการ¹ ไอยฤทธิ์ ไทยพิสุทธิกุล¹ และ ประชาติ เพิ่มพิกุล²

¹ภาควิชาจุลชีววิทยา ภาควิชาเวชศาสตร์การชนาการเลือด คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

บทนำ เลือดบริจากทุกรายต้องตรวจคัดกรองไวรัสตับอักเสบบีด้วยการตรวจหาเอนติเจนบันผิวของเชื้อไวรัสตับอักเสบบี (HBsAg) ผู้ผลิตชุดตรวจแนะนำให้ตรวจยืนยันผลการตรวจกรองก่อนสรุปผลว่าเป็นบวกทุกราย แต่การตรวจยืนยันดังกล่าวไม่ได้ถูกดำเนินการในงานประจำ เพราะในอดีตมีความซุกของการติดเชื้อไวรัสตับอักเสบบีสูงและต้องมีค่าใช้จ่ายเพิ่มเติม อย่างไรก็ตามในระยะเวลาหลายสิบปีมานี้ ความซุกของการติดเชื้อไวรัสตับอักเสบบีได้ลดลงอย่างต่อเนื่อง จากผลของการให้วัคซีนป้องกันไวรัสตับอักเสบบีในเด็กแรกเกิดซึ่งเริ่มมาตั้งแต่ปี พ.ศ. 2535 **วัตถุประสงค์** เพื่อศึกษาความถูกต้องของการตรวจยืนยันผลการตรวจของ HBsAg (HBsAg confirmatory test) แอนติบอดีต่อแgen g琅ของเชื้อไวรัสตับอักเสบบี (anti-HBc) และแอนติบอดีต่อเอนติเจนบันผิวของเชื้อไวรัสตับอักเสบบี (anti-HBs) ในผู้บริจากเลือดที่มีผลการตรวจ HBsAg เป็นบวกของโรงพยาบาลศิริราช **วัสดุและวิธีการ** นำตัวอย่างเลือดจากผู้บริจากเลือดที่มีผลการตรวจ HBsAg เป็นบวก (HBsAg Quali II, Architect, Abbott Laboratory) ระหว่างเดือนมกราคม พ.ศ. 2557 ถึง พฤษภาคม พ.ศ. 2559 จำนวน 525 ราย มาทำการตรวจยืนยัน HBsAg ด้วยวิธี neutralization (HBsAg Quali II confirmatory test, Architect, Abbott Laboratory) และตรวจหา anti-HBc, anti-HBs (Architect, Abbott laboratory) **ผลการศึกษา** ในช่วงที่ทำการศึกษาพบว่าความซุกของ HBsAg คือ ร้อยละ 0.50 แต่เมื่อทำการตรวจยืนยันใน 525 ราย การยืนยันพบว่ามี 475 ราย (ร้อยละ 90.5) ที่ให้ผลบวกและมี 50 ราย (ร้อยละ 9.5) ที่ให้ผลลบ นอกจากนี้พบว่าในกลุ่มที่ทำการตรวจยืนยันเป็นบวก ค่าลัษณะนิยมการตรวจ (s/co) HBsAg ที่สูงกว่า 300 มีความลัมพันธ์กับการทำนายผลตรวจยืนยันที่เป็นบวกทุกราย (100 % positive predictive value) เมื่อแปลผลร่วมกับ anti-HBc และ anti-HBs พบว่ามี 5 ราย (ร้อยละ 1.05) ที่ติดเชื้อในระยะแรก มี 456 ราย (ร้อยละ 96.00) ที่ติดเชื้อเรื้อรัง และที่น่าสนใจคือมี 14 ราย (ร้อยละ 2.95) ที่ตรวจพบทั้ง HBsAg และ anti-HBs ซึ่งเป็นการรายงานครั้งแรกในผู้บริจากเลือดของประเทศไทย และผู้ที่ตรวจพบเฉพาะ anti-HBs ไม่มีรายใดที่การตรวจยืนยัน HBsAg ให้ผลบวก **สรุป** ในการตรวจกรอง HBsAg ในผู้บริจากเลือดด้วยชุดตรวจ HBsAg Quali II (Architect, Abbott Laboratory) ควรทำการตรวจยืนยันผลการตรวจกรองที่เป็นบวกก็ต่อเมื่อค่าลัษณะนิยมการตรวจกรอง HBsAg s/co น้อยกว่า 300 เท่านั้นทั้งนี้เพื่อป้องกันการให้เลือดบริจากเลือดตلوดไปซึ่งเป็นสิ่งที่ไม่เหมาะสม

คำสำคัญ : ● HBsAg neutralization ● Blood donor ● Coexistence HBsAg and anti-HBs

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2562;29:101-8.

Introduction

Hepatitis B surface antigen (HBsAg) is a mandatory infectious screening test for donated blood to prevent transmission of hepatitis B virus (HBV) to the patients. In a country with prevalence of HBsAg higher than 0.4%, the HBsAg repeatedly reactive (HBsAg RR) was usually interpreted as a positive result.¹ According to the WHO report in 2017², the prevalence of HBV in Southeast Asian region is about 2-4%. However, the manufacturer instruction and guideline for donor management^{3,4} recommended to confirm every case of HBsAg RR before interpretation as a positive result. But in routine practice, the laboratory does not perform HBsAg confirmation due to additional expense and laboratory skills. This practice lead to permanently defer all blood donors who had HBsAg RR and unnecessary loss of blood donors. This also affected blood donors recruitment of blood for patients which is one of the contributing factors of insufficient blood supply. So we would like to thoroughly study about the confirmation of HBsAg. The objective of this study was to determine the HBsAg confirmation results, anti-HBc, and anti-HBs in HBsAg RR blood donor at Siriraj Hospital.

Materials and Methods

The study was approved by Siriraj IRB no. Si 614/2016. Our laboratory is accredited for the HBsAg screening test by ISO 15189 since 2010.

Blood Samples

From January 2014 to May 2016, there were 131,269 donations which 643 donations had HBsAg RR. We recruited only 525 HBsAg RR blood donors from repository EDTA plasma for the study because of the limitation of the budget. The inclusion criteria were all samples with HBsAg RR and had s/co $\leq 1,000$ and randomly selected samples with HBsAg RR s/co $> 1,000$. The number of included and excluded samples is shown in Figure 1. These samples were kept in -80°C monitoring temperature freezer until use. We performed HBsAg screening in blood donors by HBsAg Qualitative II assay (ARCHITECT[®]) on automated system ARCHITECT[®] i2000 system (Abbott Ireland, Diagnostics Division, Sligo, Ireland) according to the manufacturer's instruction. The result of HBsAg was reactive if s/co ≥ 1.00 .

HBsAg Confirmatory test

We performed HBsAg neutralization by HBsAg Qualitative II confirmatory assay test (ARCHITECT[®]) on automated system ARCHITECT[®] i2000 system (Abbott Ireland, Diagnostics Division, Sligo, Ireland) according to the manufacturer's instruction.

Detection of anti-HBc and anti-HBs

We performed anti-HBc and anti-HBs by Anti-HBc II assay (ARCHITECT[®]) and anti-HBs assay (ARCHITECT[®]), on automated system ARCHITECT[®] i2000 system (Abbott Ireland, Diagnostics Division, Sligo, Ireland) according to the manufacturer's instruction.

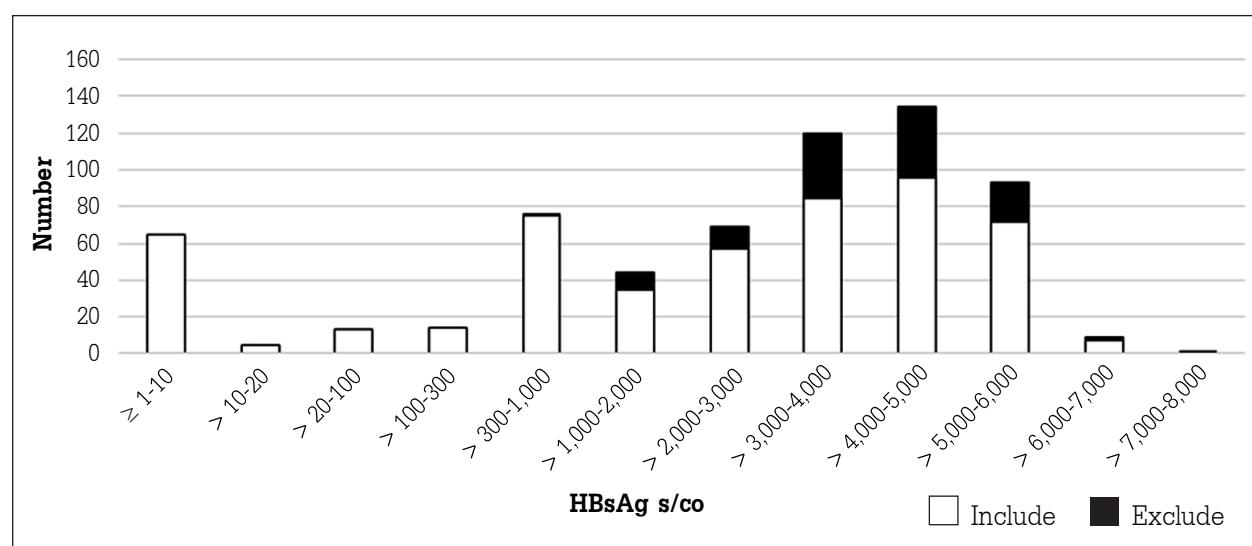
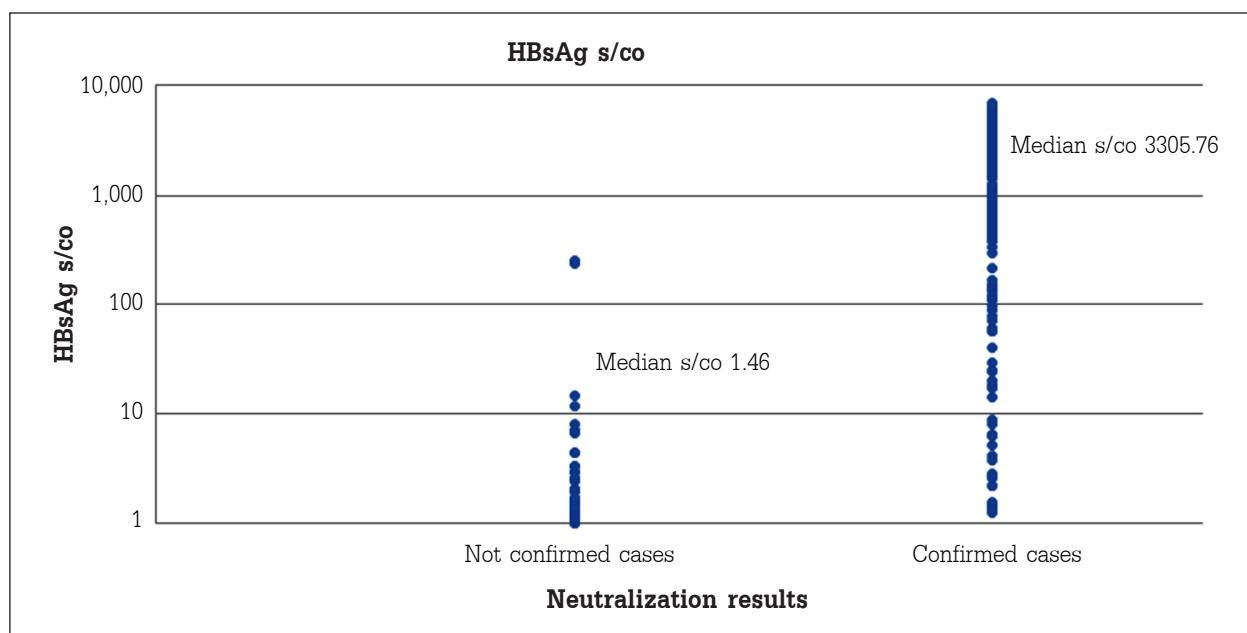


Figure 1 Distribution of HBsAg s/co of included and excluded samples

Table 1 Comparison of the demographic data between HBsAg confirmed group and not-confirmed group

	Confirmed HBsAg N = 475	Not-confirmed HBsAg N = 50	p-value
Age (year)	34.66 ± 9.23	31.18 ± 9.80	< 0.05
Birth			
Before EPI	434	35	0.000003
After EPI	41	15	
Gender			
Male	348	28	0.0031
Female	117	22	
HBsAg median s/co	3305.76	1.46	< 0.0001

**Figure 2** The distribution of HBsAg s/co and neutralization results

Analysis and statistics

We analyzed the demographic data including age, sex, the year of birth before or after 1992 which was the start of an extended program of immunization (EPI) in Thailand and the neutralization of HBsAg RR results. The statistical differences were compared by paired t-test for age, chi-square test for sex and the year of birth and Mann-Whitney U Test for the median of HBsAg s/co between 2 groups; confirmed and not confirmed groups. We used cut-off for significant results if the $p < 0.05$.

Results

The demographic data of the HBsAg RR blood donors is shown in Table 1. From 525 HBsAg RR tested samples, 475 samples (90.5%) were confirmed

by neutralization and 50 samples were not-confirmed (9.5%). The average age of the confirmed and not-confirmed group was 34.66 ± 9.23 and 31.18 ± 9.80 years, respectively, which was significantly difference ($p < 0.05$). The male to female ratio of the confirmed and not-confirmed group was 3.06 (M: F 358:117) and 1.27 (M: F 28:22) which was significantly difference ($p = 0.0031$). The results of this study showed that 434 confirmed HBsAg donors from 525 (91.36%) HBsAg RR were born before EPI implementation and 8.64% were born after the implementation of EPI which was significantly difference ($p = 0.000003$).

Figure 2 shows the distribution of HBsAg s/co and the neutralization results in confirmed group and not-confirmed group. The median HBsAg s/co of not-

Table 2 HBsAg signal (s/co), neutralization results and positive predictive value (PPV)

HBsAg signal (s/co)	Confirmed HBsAg	Not-confirmed HBsAg	PPV (%)
1.00-10.00	18	47	27.7
10.01-20.00	3	2	60.0
20.01-100.00	13	0	100
100.01-300.00	13	1	92.86
300.01-1,000.00	75	0	100
1,000.01-2,000.00	35	0	100
2,000.01-3,000.00	57	0	100
3,000.01-4,000.00	85	0	100
4,000.01-5,000.00	96	0	100
5,000.01-6,000.00	72	0	100
6,000.01-7,000.00	7	0	100
7,000.01-8,000.00	1	0	100
Total	475	50	90.5

Table 3 HBsAg signal (s/co), neutralization results and anti-HBc and anti-HBs results

Anti-HBc	Anti-HBs	HBsAg s/co ≤ 300		HBsAg s/co > 300		Total
		Confirmed HBsAg	Not-confirmed HBsAg	Confirmed HBsAg	Not-confirmed HBsAg	
Neg	Neg	3	37	2	0	42
Neg	Pos	0	7	0	0	7
Pos	Neg	39	1	417	0	457
Pos	Pos	5	5	9	0	19

Pos = positive; Neg = negative

confirmed group was 1.46 while in confirmed groups was 3305.76 that were significantly difference ($p < 0.0001$) as shown in Table 1. The entire not-confirmed group had HBsAg s/co < 20 except one sample which HBsAg s/co was 250.

Table 2 shows the signal of HBsAg, neutralized results and positive predictive value (PPV) of each interval of HBsAg value. In HBsAg s/co < 10.00, the PPV was 27.7% and increased to 60% in HBsAg s/co 10.01-20.00. At HBsAg s/co 300.01 to maximum value, the PPV was 100%. There was only one sample of HBsAg s/co at 250.00 which could not be confirmed. Repeated neutralization for this sample gave same result. We analyzed the neutralization result of a low signal of HBsAg which defines as s/co ≤ 300 and a high signal which HBsAg s/co > 300. In the low signal group which included 97 HBsAg RR, only 47 from 97 samples can be

confirmed or calculated PPV of 48.45% while all 428 of the high signal group were confirmed by neutralization or PPV of 100%.

Table 3 shows the signal of HBsAg (s/co) which we divided into 2 groups: low signal of HBsAg (s/co ≤ 300.00) and high signal (s/co > 300.00), and the results of neutralization, anti-HBc and anti-HBs. The results of anti-HBc and anti-HBs could be divided into 4 groups; group 1 as both of them were negative results, only 11.9% (5 from 42) could be confirmed HBsAg. Group 2 as anti-HBc negative, anti-HBs positive, no one could be confirmed HBsAg (0 from 7). For group 3 as anti-HBc positive, anti-HBs negative, 99.78% (456 from 457) could be confirmed HBsAg. And group 4 as both of them was positive, 73.68% (14 from 19) could be confirmed HBsAg. We found that almost 100% of the confirmed HBsAg results were related to anti-HBc positive results

(470 from 476 or 98.73%). And in a group of high signals ($s/co > 300.00$), 100% were related to anti-HBc positive results (426 from 426).

Discussion

In the period of this study, the prevalence of HBsAg RR in blood donor was 0.5% which is much lower than the previous reports on prevalence in blood donors during 1989-1994 reported by Bejracandra S. et al from our institute which was 3.7 %⁵ and from The National Blood Centre, Thai Red Cross Society which was 2.6% in 2009.⁶ From internal data of Blood Bank, Siriraj Hospital, HBsAg RR rate in blood donors gradually declined from 2.0% in 2004 to 0.5% during the study period. In our opinion, the declining prevalence was the result of systemic approach to reduce hepatitis B transmission from mother to child by establishment of HBsAg screening test in all pregnant women and extended program of immunization (EPI) for the newborn which was started in 1992. The coverage of EPI increased from 77.4% in 1994 to 98.3% in 2008.^{6,7} We did not include all HBsAg RR to be tested for neutralization due to limited budget. The selected cases were based on the signal of HBsAg because there were reports indicated about the correlation between positive confirmation by neutralization with a high signal of other HBsAg assays.⁸⁻¹⁰ Figure 1 shows the distribution of HBsAg signals to all samples which had normal distribution except for the range of low positive signal of HBsAg ($s/co 1-10$).

In 475 from selected 525 samples (90.4%) that HBsAg could be confirmed, the data showed that HBsAg s/co was higher than 300 in all samples. Our results were correlated with the finding from Shao et al¹¹ and Kiely P et al.¹² All the information suggested the need to perform the confirmation of HBsAg in low HBsAg signal group. In addition, the study also showed that the average age of blood donors who had positive confirmation of HBsAg was significantly higher than the average age of not-confirmed group as previous study.⁷

Furthermore, our study showed that 91.36% of confirmed HBsAg RR blood donors was born before EPI implementation in 1992 and 8.64% was born after EPI. This finding was correlated with study of Posuwan N et al¹³ which reported 4.5% before EPI and 0.6% after EPI implementation. This different prevalence of HBsAg between general population and blood donors reflects the effectiveness of blood donor selection and the effectiveness of EPI implementation in Thailand as well.

From the tested samples, we could estimate that all non-tested samples which HBsAg $s/co > 1,000$ will give positive HBsAg confirmation. The estimated number of positive confirmed samples should be 593 (475 tested samples plus 118 untested samples with all cases had HBsAg $s/co > 300$) from a total of 643 HBsAg RR or positive predictive value of 92.2%. We estimated the specificity of Architect HBsAg Quali II in this study was 99.96% calculated from 131,269 samples which are correlated with Popp C study⁹ and the manufacturer specificity with a range of 99.68-99.98%. And from our data, even though the specificity of the test of blood donor is high, we still need to do the confirmatory test to prevent falsely identified donor as being infected with hepatitis B and lead to unnecessary loss of blood donors.¹⁴

If we analyzed our results and look at evidence of this study from Table 1, all samples of s/co more than 300 will have positive confirm result so PPV of HBsAg signal > 300 will be 100%. Then we can interpret HBsAg RR as a positive result. This can be used as an evidence-based guide to a laboratory to select cases for HBsAg confirmation when using for blood donor screening by Abbott HBsAg Quali II. From our data, we suggest that the confirmation of HBsAg RR is needed only if HBsAg s/co was less than 300. Actually, in the not-confirmed group, 49 from 50 had HBsAg $s/co < 20$, only one sample with HBsAg s/co 250 had negative neutralization result. We did neutralization in neat and in dilution of 1:20 which still had gave a reactive result (s/co 10-12 but cannot neutralize with anti-HBs in the kit) after further

1:500 dilution, the result was non-reactive. This donor is a 22-year-old female and had both negative anti-HBc and anti-HBs. This sample gave a non-reactive result from different HBsAg tests kit (Elecsys® HBsAg II, Roche Diagnostics). Unfortunately, we cannot contact this donor for further test. Our interpretation is false reactive but mutation of HBV could not be excluded.¹⁵ From this outlier, we decided to choose proper selective of HBsAg signal > 300 .

The interpretation of anti-HBc and anti-HBs results showed that 1.05% of HBsAg confirmed case was an acute infection which had negative both anti-HBc and anti-HBs¹⁵, 96% were chronic infection which had positive anti-HBc but negative anti-HBs. Interestingly, we found 14 donors (2.95%) that had coexistence of confirmed HBsAg together with anti-HBs. This is the first report on coexistence of HBsAg and anti-HBs in blood donors from Thailand. This finding is not uncommon and was reported in several studies which the rate varied from 2.5-5% and 2.5-30% in chronic hepatitis B patients¹⁷⁻²² and may have clinical significance as reported.^{23,24} The rate of this finding correlates with the observed rate of 2.5-5% in population screening.¹⁸⁻²⁰ The clinical significance of this finding was explored by several studies and seemed to relate to long lasting immune clearance and favorable selection of oncogenic potential variant of HBV quasispecies.²⁵ Further study in this group of HBsAg positive blood donor is needed to better identify clinical importance.

Conclusion

The prevalence of HBsAg repeatedly reactive in blood donors at Siriraj Hospital during 2014-2016 was 0.5%. The HBsAg was confirmed by neutralization in 90.5% of HBsAg RR. All HBsAg RR with s/co > 300 could be confirmed by neutralization but only 48.45% of HBsAg RR s/co ≤ 300 could be confirmed. So we recommend that only HBsAg RR s/co ≤ 300 should be confirmed. In addition, we found coexistence of confirmed HBsAg with anti-HBs which is the first report on blood donors in Thailand.

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