Am J Transl Res 2021;13(8):9771-9777
www.ajtr.org /ISSN:1943-8141/AJTR0132565

Original Article

The utility of grey zone testing in improving blood safety

Min Wang*, Shu-Hong Yu*, Zong-Zheng Han

*Blood Transfusion Department, Hanchuan People's Hospital, Hanchuan 431600, Hubei Province, China. *Equal contributors.*

Received February 27, 2021; Accepted April 19, 2021; Epub August 15, 2021; Published August 30, 2021

Abstract: Object: Transfusion-transmitted infections threaten blood safety. The management of samples with weak reactivity is confusing. Our study aimed to investigate the utility of grey zone (GZ) testing in the screening of the hepatitis B virus (HBV), the hepatitis C virus (HCV), and the human immunodeficiency virus (HIV). Methods: Chemiluminescence assays (CLA) were used for the blood screening. For each infectious marker, two CLA kits were used, but for each sample, only one kit was used for each infectious marker. An S/CO ≥ 8.0 was considered reactive, and an S/CO < 0.8 was considered non-reactive. A 0.8 ≤ S/CO < 1.0 was set as GZ1, a 1.0, ≤ S/CO < 4.0 was set as GZ2, and a 4.0 ≤ S/CO < 8.0 was set as GZ3. The samples located at different GZs were separately verified using nucleic acid testing (NAT). Results: 22,081 patients requiring blood transfusions were included in this study. The cohort had an average age of 32.6 ± 10.6 years old. HBV test kit B (0.41% reactivity and 0.58% GZ rate) was more sensitive than kit A (0.28% reactivity and 0.43% GZ rate). HCV test kit A (0.29% reactivity and 0.57% GZ rate) was more sensitive than kit B (0.27% reactivity and 0.31% GZ rate). HIV test kit A (0.10% reactivity and 0.20% GZ rate) was more sensitive than kit B (0.08% reactivity and 0.11% GZ rate). All the samples in GZ1 were negative for NAT. HBV test kit A has negativities of 20.00% and 4.35% in GZ2 and GZ3, respectively, while HBV test kit B has negativities of 36.84% and 35.48% in GZ2 and GZ3, respectively. HCV test kit A has negativities of 100.00% and 66.67% in GZ2 and GZ3, respectively, while HCV test kit B has negativities of 72.73% and 27.78% in GZ2 and GZ3, respectively. HIV test kit A has negativities of 100.00% and 88.24% in GZ2 and GZ3, respectively, while HCV test kit B has negativities of 100.00% and 50.00% in GZ2 and GZ3, respectively. Conclusion: The GZ is useful in blood screening for HBV, HCV, and HIV. A test kit with a high sensitivity has a low specificity. Different test kits should be set with different GZs based on their sensitivity and specificity.

Keywords: Hepatitis B virus, hepatitis C virus, human immunodeficiency virus, nucleic acid testing, grey zone

Introduction

The hepatitis B virus (HBV), the hepatitis C virus (HCV), and the human immunodeficiency virus (HIV) are highly infectious through blood and are among the most common infectious diseases. According to statistical data from the Division of Communicable Disease Prevention and Management, which belongs to the Ministry of Health of the People's Republic of China, there are 1,002,000 HBV patients, 224,000 HCV patients, and 71,000 HIV patients in China [1]. There are also several virus carriers [2]. Blood samples from the virus carriers may induce transfusion-transmitted infections (TTIs), crucially threatening the safety of blood transfusions. It has been reported that the probability of TTIs is more than 1/60,000 [3-5], indicating the necessity of screening for infectious diseases to prevent TTIs in blood collection. Nowadays, serological testing and nucleic acid testing (NAT) are commonly used for infectious markers screening worldwide [6]. In China, serological testing is the standard method in primary screening, and NAT is used for validation [7].

Serological testing generally includes enzyme-linked immunosorbent assays (ELISA), gold immune chromatography assays (GICA), and chemiluminescence assays (CLA) [8]. Compared to ELISA and GICA, CLA is more sensitive and therefore can better analyze the blood samples from the individuals who are recessively infectious or who are in the "window period" [9-11]. Contributing a great improvement to the safety of clinical transfusion, to epidemiological research, and to the treatment and observation of the infectious viral diseases, CLA has been vigorously promoted in China [12, 13].

However, with advances in sensitivity, the specificity of CLA may be unsatisfactory, especially for some weakly-reactive samples [14].

The analysis of CLA results is based on the ratio of the sample's luminescence (S) and a cut-off value (CO). Theoretically, a sample is negative with an S/CO that is less than 1, otherwise, the sample is positive. However, in practical application, many samples are weakly reactive with S/CO values nearly 1 and have a poor repeatability [15]. Judging the samples with weak reactivity is confusing. To overcome this problem, the grey zone (GZ) was introduced in the CLA. S/CO ratios falling in the GZ will be further validated using NAT, a sensitive and specific, but costly, method. Previous studies have reported the application of GZ ranging from 0.9 to 1 in preventing TTI, however, the results contradict each other [16, 17]. Therefore, the utility of grey zone testing strategy in improving blood safety is not yet clear.

In this study, we established a series of GZs to detect the utility of GZ testing in the screening of HBV, HCV, and HIV using CLA, and the samples in the GZs were verified by NAT.

Materials and methods

Blood samples

A total of 22,081 blood samples were collected from patients requiring blood transfusions from 2017 to 2020. Human samples involved in this study were managed using protocols approved by the Ethical Committee of the Hanchuan People's Hospital (E2017010). Informed consents were obtained from all the patients.

Serum separation

Serum separation was performed as described previously [18]. Whole blood samples were collected in 1.5 ml tubes and incubated at 4°C overnight. Then the samples were centrifuged at 2000 rpm for 10 min. The serum on the upper layer was transferred to a new tube and stored at -80°C.

Chemiluminescence assay (CLA)

For the HBV screening, MAGLUMI HBsAg IgG CLA Kits (Haifei Biotech, named as HBV test kit A) and HBsAg quantitative Elecsys Kits (Roche Biotech, here called HBV test kit B) were used. For the HCV screening, MAGLUMI HCV IgG CLA Kits (Haifei Biotech, here called HBV test kit A) and HCV IgG CLA Kits (Chemclin Diagnosis, here called HBV test kit B) were used. For the HIV screening, MAGLUMI HIV IgG CLA Kits (Haifei Biotech, here called HBV test kit A) and HIV Ab CLA Kits (Chemclin Diagnosis, here called HBV test kit B) were used. The CLA procedure was described previously [19]. Briefly, 50 µl of sample serum was mixed with an equal volume sample diluent, and the mixture was added to the well. The plate was incubated at 37°C for 30 min, and then was washed 5 times. 100 µl enzyme conjugate was added to each well except for the blank ones. The plate was incubated at 37°C for 30 min, and then washed 5 times. Luminescent substrate A and B were mixed at the ratio of 1:1, and 100 µl mixture was added to each well. The plate was incubated at room temperature for 5 min, and the relative luminescence unit (RLU) was analyzed using Chemclin-600 (Chemclin Diagnosis) within 30 min. The CO value of each kit was calculated based on the negative sample's RLU according to the relative protocol.

Nucleic acid testing (NAT)

NAT was performed as described previously [20] with an HBV, HCV, and HIV (1 + 2 types) Nucleic Acid Testing Kit (Sansure Biotech). In brief, nucleic acid extraction reagent, carrier RNA, and an internal standard were mixed. The mixture, washing buffer A, washing buffer B, a mixture of eluent and specific beads were loaded to the relative positions on Natch S (Sansure Biotech), respectively. The blood samples were loaded to Natch S and nuclei extraction was performed automatically. The treated samples were mixed with reagent A, reagent B, reverse transcriptase, RNA protects, and Taq polymerase and loaded onto a SLAN-96P (Sansure Biotech) to perform RT-qPCR. Samples with a cycle threshold (Ct) less than 45 were considered positive, and the others were considered negative. The test limit was 3 IU/ml for HBV, 10 IU/ml for HCV, and 45 IU/ml for HIV.

Screening procedure

The strategy for the infectious marker screening is shown in **Figure 1**. Briefly, each blood sample was analyzed for HBV surface antigens (HBsAg), HCV antibodies and HIV antibodies.

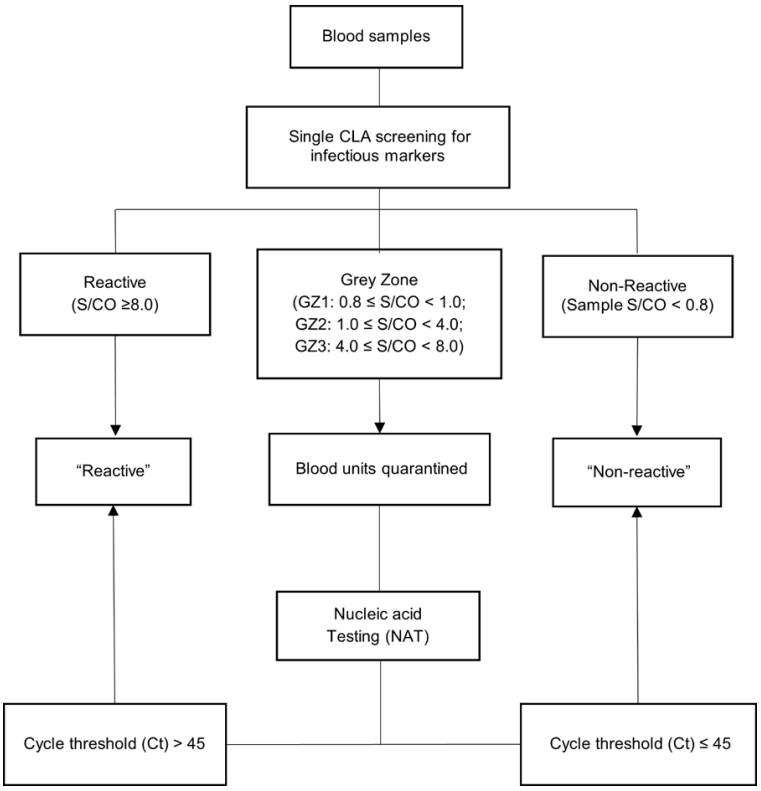

Figure 1. The screening procedure used in the study.

Table 1. Basic information about the patients requiring blood transfusions

Variable	Statistics
Age (Years)	42.6 ± 15.6
Sex	
Male	11498
Female	10583
Height (cm)	173.1 ± 13.7
Weight (kg)	65.6 ± 7.2
Blood type	
A	6050
B	7286
AB	2562
O	6183

For each infectious marker, two CLA kits were used, but for each sample, only one kit was used for each infectious marker. An S/CO ≥ 8.0 was considered reactive, and an S/CO < 0.8 was considered non-reactive. 0.8 ≤ S/CO < 1.0 was set as GZ1, 1.0 ≤ S/CO < 4.0 was set as GZ2, and 4.0 ≤ S/CO < 8.0 was set as GZ3. Samples located at different GZs were verified using NAT separately.

Statistical analysis

The data are presented as the mean ± SD or the number (present). The statistical analysis was performed using SPSS 18.0 software. The comparisons of the gene frequencies among the different groups were performed using Fisher's exact test.

Result

Primary screening for HBsAg, HCV antibodies, and HIV antibodies

22,081 patients requiring blood transfusions, of which 11,498 (52.07%) were male and 10,583 were female, were included in this study. The patients' ages ranged from 18 to 50 years old (32.6 ± 10.6 years). Detailed information about the cohort is shown in **Table 1**.

For the HBsAg detection (**Table 2**), 10,697 samples were analyzed using HBV test kit A, of which 10,621 (99.29%) were non-reactive, 30 (0.28%) were reactive, and 46 (0.43%) were in the GZ (13 were in GZ1, 10 were in GZ2, and 23 were in GZ3). Also, 11,384 were analyzed using HBV test kit B, of which 11,270 (99.00%) were non-reactive, 47 (0.41%) were reactive, and 67 (0.58%) were in the GZ (17 were in GZ1, 10 were in GZ2, and 23 were in GZ3). The data indicated that kit B is more sensitive than kit A.

For the HCV antibody detection (**Table 2**), 9,965 samples were analyzed using HCV test kit A, of which 9879 (99.14%) were non-reactive, 29 (0.29%) were reactive, and 57 (0.57%) were in the GZ (6 were in GZ1, 21 were in GZ2, and 30 were in GZ3). Also, 12,116 were analyzed using HCV test kit B, of which 12,045 (99.41%) were non-reactive, 33 (0.27%) were reactive, and 38 (0.31%) were in the GZ (9 were in GZ1, 11 were in GZ2, and 18 were in GZ3). The data revealed that kit A is more sensitive than kit B.

For the HIV antibody detection (**Table 2**), 12,531 samples were analyzed using HIV test kit A, of which 12,493 (99.70%) were non-reac-

Table 2. Primary screening results for HBsAg, HCV antibodies, and HIV antibodies

Infectious marker	Test Kit	Sample number	Non-reactive (%)	Reactive (%)	Grey zone			
					GZ1 (%)	GZ2 (%)	GZ3 (%)	Total GZ (%)
HBV	A	10697	10621 (99.29)	30 (0.28)	13 (0.12)	10 (0.09)	23 (0.22)	46 (0.43)
	B	11384	11270 (99.00)	47 (0.41)	17 (0.15)	19 (0.17)	31 (0.27)	67 (0.58)
HCV	A	9965	9879 (99.14)	29 (0.29)	6 (0.06)	21 (0.21)	30 (0.30)	57 (0.57)
	B	12116	12045 (99.41)	33 (0.27)	9 (0.07)	11 (0.09)	18 (0.15)	38 (0.31)
HIV	A	12531	12493 (99.70)	13 (0.10)	3 (0.02)	5 (0.04)	17 (0.13)	25 (0.20)
	B	9550	9531 (99.80)	8 (0.08)	3 (0.03)	2 (0.02)	6 (0.06)	11 (0.11)

Table 3. NAT verification results of GZ1

Infectious marker	Test Kit	GZ1			
		Positive (%)	Negative (%)	*P* value	Significance
HBV	A	0 (0.00)	13 (100.00)	P>0.9999	n.s.
	B	0 (0.00)	17 (100.00)		
HCV	A	0 (0.00)	6 (100.00)	P>0.9999	n.s.
	B	0 (0.00)	9 (100.00)		
HIV	A	0 (0.00)	3 (100.00)	P>0.9999	n.s.
	B	0 (0.00)	3 (100.00)		

n.s.: no significance.

Table 4. NAT verification results of GZ2

Infectious marker	Test Kit	GZ2			
		Positive (%)	Negative (%)	*P* value	Significance
HBV	A	8 (80.00)	2 (20.00)	P=0.4311	n.s.
	B	12 (63.16)	7 (36.84)		
HCV	A	0 (0.00)	21 (100.00)	P=0.3098	n.s.
	B	3 (27.27)	8 (72.73)		
HIV	A	0 (0.00)	5 (100.00)	P>0.9999	n.s.
	B	0 (0.00)	2 (100.00)		

n.s.: no significance.

tive, 13 (0.10%) were reactive, and 25 (0.20%) were in the GZ (3 were in GZ1, 5 were in GZ2, and 17 were in GZ3). Also, 9,550 were analyzed using HIV test kit B, of which 9531 (99.80%) were non-reactive, 8 (0.08%) were reactive, and 11 (0.11%) were in the GZ (3 were in GZ1, 2 were in GZ2, and 6 were in GZ3), indicating that kit A is more sensitive than kit B.

NAT verification of the GZs

All the samples in the GZs were verified using NAT. For GZ1 ($0.8 \leq S/CO < 1.0$), all the samples in GZ1 were negative for all three infectious markers (**Table 3**).

Of the 10 samples located in GZ2 which were analyzed using HBV test kit A, 2 (20%) were negative, and of the 19 samples located in GZ2 which were analyzed using HBV test kit B, 7 (36.84%) were negative (**Table 4**). There was no significant difference between the validated results of the two kits (P=0.4311) (**Table 4**). In the 21 samples located in GZ2 which were analyzed by HCV test kit A, all were negative, and in the 11 samples located in GZ2 which were analyzed using HCV test kit B, 8 (72.73%) were negative (**Table 4**). Although the false-positive rate of HCV test kit A was higher, there was no significant difference between the validated results of the two kits (P=0.3098) (**Table 4**). Of the 7 samples located in GZ2 which were analyzed by both HIV test kits, all were negative (**Table 4**).

In the 23 samples located in GZ3 which were analyzed by HBV test kit A, 1 (4.35%) were negative, and in the 31 samples located in GZ3 which were analyzed by HBV test kit B, 11 (35.48%) were negative (**Table 5**). The false-positive rate of HBV test kit B was significantly higher than the false-positive rate of HBV test kit A (P=0.0078) (**Table 5**). Of the 30 samples located in GZ3 which were analyzed using HCV test kit A, 20 (66.67%) were negative, and of the 18 samples located in GZ3 which were analyzed using HCV test kit B, 5 (27.78%) were negative (**Table 5**). The false-positive rate of HCV test kit A was significantly higher than the false-positive rate of HCV kit B (P=0.0162) (**Table 5**). In the 17 samples located in GZ3 which were analyzed using HIV test kit A, 15 (88.24%) were negative, and

Table 5. NAT verification results of GZ3

Infectious marker	Test Kit	GZ3			
		Positive (%)	Negative (%)	*P* value	Significance
HBV	A	22 (95.65)	1 (4.35)	P=0.0078	**
	B	20 (64.52)	11 (35.48)		
HCV	A	10 (33.33)	20 (66.67)	P=0.0162	*
	B	13 (72.22)	5 (27.78)		
HIV	A	2 (11.76)	15 (88.24)	P=0.0886	n.s.
	B	3 (50.00)	3 (50.00)		

n.s.: no significance; *P < 0.05; **P < 0.01.

in the 6 samples located in GZ3 which were analyzed using HBV test kit B, 3 (50.00%) were negative (**Table 5**). Although the false-positive rate of HIV test kit A was higher, there was no significant difference between the validated results of the two kits (P=0.0886) (**Table 5**).

Discussion

Our study investigated the utility of GZ testing in the screening of HBsAg, HCV antibodies, and HIV antibodies using different CLA kits. A total of 22,081 patients requiring blood transfusions were included in this study. We found that GZ1 might be useless because all the samples with S/CO located in this interval were proved negative for all three infectious diseases by NAT. GZ2 was helpful in the screening of HBV because more than 20% of the samples with S/CO located in this interval were proved negative for HBV. GZ2 might be helpful in the screening of HCV with the use of HCV test kit B, and although the majority (72.73%) of the samples were negative, GZ2 might be useless in the screening of HIV because all the samples with S/CO located in this interval were proved negative for HIV. GZ3 was useful for the screening of all three infectious diseases because only a part of the samples with S/CO located in this interval were proved negative by NAT.

Archana Solanki, et al. [16] reported that a GZ ranging from 0.9 to 1 was useful for the screening of HBV, HCV, and HIV using ELISA in 2016, while Gunjan Bhardwaj, et al. [17] reported that the same GZ was useless because all the samples in GZ were proved negative for HBV, HCV, and HIV by confirmatory tests in 2020. Our GZ1 data agrees with Gunjan Bhardwaj's study. Those contrary reports might be explained by an improvement in the testing techniques. However, this conclusion needs more careful validation with more test kits and a larger study cohort providing more blood samples.

HBV can induce acute and chronic hepatitis, liver cirrhosis, and liver cancer [21-24]. The level of HBsAg in the blood increases in the first few weeks after HBV infection, indicating that blood samples from people in the "window period" might be weakly reactive for HBsAg detection [25]. In our study, more than 50% of the samples with S/CO located in GZ2 and GZ3 were positive for HBV NAT, indicating that the installation of a GZ of more than 1 could promote the screening of infectious blood samples from healthy ones, and thus improve transfusion blood safety.

For the primary screening of HCV and HIV antibodies, nearly all the samples with S/CO located in GZ2 were found negative using NAT, and this agrees with previous studies that reported a high false positivity in HCV and HIV antibody testing [26, 27]. The high false positivity might be induced by a high concentration of un-specific IgG, rheumatoid factors, complements, and self-targeted antibodies. However, the factors inducing the high false positivity in the screening of HCV and HIV antibodies are not verified, and further studies to improve the specificity are warranted.

It is worth noting that different kits have different sensitivities and specificities. HBV test kit B is more sensitive than HBV test kit A, but its false positivity is higher. HCV test kit A is more sensitive than HCV test kit A, but with a higher false positivity. Even though there was no statistical difference between the different HBV or HCV screening kits in the GZ2 group, this might be induced by the small size of the study cohort. Generally, a kit with a high sensitivity must have a low specificity, as previous studies agree [28, 29], and shedding light on the set of GZs varies depending on the sensitivity and specificity of the test kit.

In conclusion, our data indicate the importance of the GZs in blood screening for HBV, HCV, and HIV. The establishment of appropriate GZs could be greatly helpful for the separation of infectious blood samples with weak reactivity

from the healthy ones, thus improving blood safety for transfusions. However, different test kits should be set with different GZs based on their sensitivity and specificity. Furthermore, more studies are needed for the optimization of GZ installation, and a larger study cohort or a multiple-center study may be helpful.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zong-Zheng Han, Blood Transfusion Department, Hanchuan People's Hospital, No. 1, Renmin Avenue, Hanchuan, Hubei Province, China. Tel: +86-15007292521; E-mail: hanzongzheng692@163.com

References

[1] Yu S, Yu C, Li J, Liu S, Wang H and Deng M. Hepatitis B and hepatitis C prevalence among people living with HIV/AIDS in China: a systematic review and Meta-analysis. Virol J 2020; 17: 127.

[2] Zhou X, Nakashima K, Ito M, Zhang X, Sakai S, Feng C, Sun H, Chen H, Li TC and Suzuki T. Prevalence and viral loads of polyomaviruses BKPyV, JCPyV, MCPyV, TSPyV and NJPyV and hepatitis viruses HBV, HCV and HEV in HIV-infected patients in China. Sci Rep 2020; 10: 17066.

[3] Ehsan H, Wahab A, Shafqat MA, Sana MK, Khalid F, Abdullah SM, Jaan A, Sheikh MM, Muneeb A, Ehsan S, Khan AY, Iftikhar R and Anwer F. A systematic review of transfusion-transmissible infections among blood donors and associated safety challenges in Pakistan. J Blood Med 2020; 11: 405-420.

[4] Kebede E, Getnet G, Enyew G and Gebretsadik D. Transfusion transmissible infections among voluntary blood donors at dessie blood bank, northeast ethiopia: cross-sectional study. Infect Drug Resist 2020; 13: 4569-4576.

[5] Steele WR, Dodd RY, Notari EP, Haynes J, Anderson SA, Williams AE, Reik R, Kessler D, Custer B and Stramer SL; Transfusion-Transmissible Infections Monitoring System (TTIMS). HIV, HCV, and HBV incidence and residual risk in US blood donors before and after implementation of the 12-month deferral policy for men who have sex with men. Transfusion 2021; 61: 839-850.

[6] Mbanya D. Use of quality rapid diagnostic testing for safe blood transfusion in resource-limited settings. Clin Microbiol Infect 2013; 19: 416-421.

[7] Rai P, Kumar BK, Deekshit VK, Karunasagar I and Karunasagar I. Detection technologies and recent developments in the diagnosis of COVID-19 infection. Appl Microbiol Biotechnol 2021; 105: 441-455.

[8] Reilly NR, Husby S, Sanders DS and Green PHR. Coeliac disease: to biopsy or not? Nature reviews. Nat Rev Gastroenterol Hepatol 2018; 15: 60-66.

[9] Pathak S and Chandrashekhar M. Transfusion transmittable infections - seroprevalence among blood donors in a tertiary care hospital of Delhi. Asian J Transfus Sci 2013; 7: 116-118.

[10] Yang B, Shi L, Lei J, Li B and Jin Y. Advances in optical assays for detecting telomerase activity. Luminescence 2019; 34: 136-152.

[11] Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol 2020; 94: 651-715.

[12] Chen C, Zhang HJ, Zhai HL, Chen Y, Han B, Li Q, Xia FZ, Wang NJ and Lu YL. Association between blood cadmium and vitamin D levels in the Yangtze Plain of China in the context of rapid urbanization. Chin Med J (Engl) 2020; 134: 53-59.

[13] Lin W, Li Y, Chen D, Yao Z, Xu H, Chen Y, Xiao J, Feng P and Gan W. High efficiency and problems of chemiluminescence assay-detected aldosterone-to-renin ratio in practical primary aldosteronism screening. Int J Hypertens 2020; 2020: 3934212.

[14] Gomaa MR, Kandeil A, Mostafa A, Roshdy WH, Kayed AE, Shehata M, Kutkat O, Moatasim Y, El Taweel A, Mahmoud SH, Kamel MN, Abo Shama NM, El Sayes M, El-Shesheny R, Bakheet OH, Elgohary MA, Elbadry M, Nassif NN, Ahmed SH, Abdel Messih IY, Kayali G and Ali MA. Prevalence of severe acute respiratory syndrome coronavirus 2 neutralizing antibodies in egyptian convalescent plasma donors. Front Microbiol 2020; 11: 596851.

[15] Seth R, Ribeiro M, Romaschin A, Scott JA, Manno M, Scott JA, Liss GM and Tarlo SM. Occupational endotoxin exposure and a novel luminol-enhanced chemiluminescence assay of nasal lavage neutrophil activation. J Allergy Clin Immunol 2011; 127: 272-275.

[16] Solanki A, Singh A and Chaudhary R. Impact of grey zone sample testing by enzyme-linked immunosorbent assay in enhancing blood safety: Experience at a tertiary care hospital in North India. Asian J Transfus Sci 2016; 10: 71-74.

[17] Bhardwaj G, Tiwari AK, Arora D, Aggarwal G, Pabbi S and Setya D. Utility of grey zone testing strategy in transfusion transmissible infection testing in blood bank is of limited value! Indian J Pathol Microbiol 2020; 63: 255-257.

[18] Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadóttir E, Schoos AM, Wolsk HM, Pedersen TM, Vinding RK, Thorsteinsdóttir S, Følsgaard NV, Fink NR, Thorsen J, Pedersen AG, Waage J, Rasmussen MA, Stark KD, Olsen

SF and Bønnelykke K. Fish oil-derived fatty acids in pregnancy and wheeze and asthma in offspring. N Engl J Med 2016; 375: 2530-2539.

[19] Zheng C, Zeng H, Lin H, Wang J, Feng X, Qiu Z, Chen J-A, Luo J, Luo Y, Huang Y, Wang L, Liu W, Tan Y, Xu A, Yao Y and Shu W. Serum microcystin levels positively linked with risk of hepatocellular carcinoma: a case-control study in southwest China. Hepatology 2017; 66: 1519-1528.

[20] Tang Z, Choi G, Nouri R and Guan W. Loop-mediated isothermal amplification-coupled glass nanopore counting toward sensitive and specific nucleic acid testing. Nano Lett 2019; 19: 7927-7934.

[21] Lebossé F, Testoni B, Fresquet J, Facchetti F, Galmozzi E, Fournier M, Hervieu V, Berthillon P, Berby F, Bordes I, Durantel D, Levrero M, Lampertico P and Zoulim F. Intrahepatic innate immune response pathways are downregulated in untreated chronic hepatitis B. J Hepatol 2017; 66: 897-909.

[22] Chou R, Blazina I, Bougatsos C, Holmes R, Selph S, Grusing S and Jou J. Screening for hepatitis B virus infection in nonpregnant adolescents and adults: updated evidence report and systematic review for the US preventive services task force. JAMA 2020; 324: 2423-2436.

[23] Lu F, Shah PA, Rao A, Gifford-Hollingsworth C, Chen A, Trey G, Soryal M, Talat A, Aslam A, Nasir B, Choudhry S, Ishtiaq R, Sanoff H, Conteh LF, Noonan A, Hu KQ, Schmidt C, Fu M, Civan J, Xiao G, Lau DT and Lu X. Liver cancer-specific serine protease inhibitor Kazal is a potentially novel biomarker for the early detection of hepatocellular carcinoma. Clin Transl Gastroenterol 2020; 11: e00271.

[24] Wang H, Wen B, Chang X, Wu Q, Wen W, Zhou F, Guo Y, Ji Y, Gu Y, Lai Q, He Q, Li J, Chen J and Hou J. Baveno VI criteria and spleen stiffness measurement rule out high-risk varices in virally suppressed HBV-related cirrhosis. J Hepatol 2021; 74: 584-592.

[25] Yang R, Cui L, Liu Y, Cong X, Fei R, Wu S and Wei L. A hook-effect-free homogeneous light-initiated chemiluminescence assay: is it reliable for screening and the quantification of the hepatitis B surface antigen? Ann Transl Med 2020; 8: 606.

[26] Ghanem KG. Management of adult syphilis: key questions to inform the 2015 centers for disease control and prevention sexually transmitted diseases treatment guidelines. Clin Infect Dis 2015; 61 Suppl 8: S818-S836.

[27] Li Y, Zhao L, Geng N, Zhu W, Liu H and Bai H. Prevalence and characteristics of hepatitis C virus infection in Shenyang City, Northeast China, and prediction of HCV RNA positivity according to serum anti-HCV level: retrospective review of hospital data. Virol J 2020; 17: 36.

[28] Liang G, Man Y, Li A, Jin X, Pan L and Liu X. Chemiluminescence assay for detection of 2-hydroxyfluorene using the G-quadruplex DNAzyme-HO-luminol system. Mikrochim Acta 2017; 185: 54.

[29] Yang X, Zhao J, Chen S, Huang Y and Zhaok S. An ultrasensitive microchip electrophoresis chemiluminescence assay platform for detection of trace biomolecules. J Chromatogr A 2020; 1613: 460693.