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(REVIEW ARTICLE)

The role of CMIA as hepatitis B screening tool in blood donors

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Abstract

Introduction: Hepatitis B infection has become an endemic problem in various nations. Numerous types of screening instruments have been identified and put to use. In comparison to other screening methods, the chemiluminescent microparticle immunoassays method is said to have a high specificity and sensitivity. It has also been reported that this technique can identify occult infections, which are difficult to identify early on. This research aims to compare the best screening techniques for detecting Hepatitis B infection.

Method: We searched PubMed, ScienceDirect databases from inception through 2000 for peer reviewed articles (in all languages) evidence related to the use of CMIA for detecting HbsAg.

Results: The CMIA method successfully identified an infection that the ELISA method was unable to detect. The CMIA kit outperformed the ELISA kits in terms of sensitivity and specificity by distinguishing true-positive HBsAg samples from those having HBsAg levels lower than the ELISA kit's grey zones.

Conclusion: The CMIA detects HBsAg in its early stages, shortening the "window period". This is critical because the viral antigens are difficult to detect during the window period because it is still early in the cycle. The identification of anti-HBc is also critical for early detection of Hepatitis B infection. The Wantai CMIA is suitable for screening blood donors because of its excellent sensitivity and specificity.

Keywords: Hepatitis B; CMIA; HbsAg

1. Introduction

Approximately 887,000 fatalities globally in 2015 were attributed to hepatitis B virus (HBV) infection. Over 257 million individuals worldwide are infected with HBV. Acute and chronic hepatitis, cirrhosis, and hepatocellular cancer are among the severe health consequences that could result from an HBV infection. HBV infection via blood transfusions continues to be a serious problem in transfusion practice. The significant rate of residual risk associated with transfusion-transmissible HBV is indicative of the virus's widespread distribution. Ever since the early 1970s, extensive HBV screening programs for blood donors have been put in place globally due to the significance of preventing the spread of HBV through blood transfusions (1).

The marker HBV surface antigen (HBsAg), which is widely produced during active infection, may be detected serologically and is a common and crucial screening procedure for blood donor samples. Though the lack of the antigen does not totally rule out the presence of the virus, there is still a chance that HBV will be transmitted during transfusion from HBsAg(−) donors. The HBsAg serological test may yield a "non-reactive" result in cases of occult infections, where viral antigens are not detectable, or during the window period of the illness (early acute phase or late chronic phase).

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As a result, testing is done for an additional seromarker, antibody against the HBV core antigen (anti-HBc). Anti-HBc serological reactivity may signal ongoing HBV exposure, a persistent infection, or an infection that is healing (1). HBV DNA may be the only sign that can be found in the early stages of acute infection. Occult HBV infection (OBI) is characterised by the presence of HBV DNA in the liver or plasma, but the absence of serum HBsAg as measured by currently available assays; anti-HBsAg and/or anti-HBc antibodies may be present. Improved HBsAg detection is especially crucial for the early identification of acute and OBI (2). Rapid diagnostic test (RDT) formats such as lateral flow, flow through, or simple agglutination assays can be used to detect HBsAg. Traditional radioimmunoassays (RIA) and enzyme immunoassays (EIA) are laboratory-based immunoassays used to detect HBsAg. More recent technologies, such as electrochemiluminescence immunoassays (ECLIA), microparticle enzyme immunoassays (MEIA), and chemiluminescent microparticle immunoassays (CMIA), use signal amplification to provide quantitative measurements (3).

Screening for HbsAg also takes part in detecting occult hepatitis B infection. The term "occult hepatitis B virus infection" (OBI) refers to the presence of replication-competent HBV DNA in the liver and/or blood of people who, according to currently available assays, test negative for hepatitis B surface antigen (HBsAg). OBI can be classified as seropositive with positive hepatitis B core antibody (anti-HBc) and/or hepatitis B surface antibody (anti-HBs) based on the HBV-specific antibody profile, or seronegative with negative anti-HBc and anti-HBs. Although the description of OBI is very clear, there is currently no common worldwide algorithm for OBI detection. Testing for HBV using HBs-Ag was the first assay that became mandatory for all blood banks globally. The screening assay's sensitivity is still a critical issue with multiple factors to consider. In certain rural locations with limited resources and low-sensitivity serological assays, OBI prevalence may be calculated inaccurately, resulting in an overestimation of OBI carriers and an underestimation of HBs-Ag presence (4).

This systematic review aims to compile, evaluate, and compare the available scientific data about the usage of CMIA in Hepatitis B infection detection to those of other available diagnostic techniques. Our goal is to provide thorough information about the use of the CMIA method, which can be used to detect Hepatitis B infection in blood donors and lower the risk of transmission during the seroconversion period. We also hope to provide information regarding the diagnosis of occult Hepatitis B, in which HBsAg may not be detectable.

2. Methods

We searched PubMed, ScienceDirect databases from inception through 2000 for peer reviewed articles (in all languages) evidence related to the use of CMIA for detecting HbsAg. We used the phrases “PubMed ((Chemiluminescent Microparticle Immunoassay OR CMIA) AND (HbsAg OR Hepatitis B Surface Antigen) AND (Blood donor OR Transfusion)); ScienceDirect with the keyword (CMIA AND HbsAg OR Hepatitis B infection). Reference list from articles identified by the search, as well as key review articles conducted by author and we did not impose any language or other restrictions on the beginning of searches.

2.1. Study selection

Our search generated a list of abstracts. Any uncertainty on the eligibility of the studies that was based on tittle and abstract made the reviewers read full paper. The study flow diagram was shown in Flowchart 1.

To be considered for inclusion, studies must explicitly define and describe the study population, the interventions, and outcomes. For the proposed comparative effectiveness review, the population of interest includes healthy adult blood transfusion donors who may or not may have their blood checked before with other screening tools besides the CMIA test. The population of interest excludes patients who are chronically ill, active hepatitis B infection, pregnant women, and children. To be considered for inclusion, clinical research studies must evaluate the specificity and sensitivity of the CMIA test in comparison to other Hepatitis B screening tests.

Table 1 Article Inclusion and Exclusion Criteria

	Inclusion Criteria	**Exclusion Criteria**
Types of studies	Controlled clinical trials (randomized control trials), observational studies	Did not describe the specificity of CMIA test on blood donors Did not describe the sensitivity of CMIA test on blood donors Did not elaborate the comparison of CMIA and other screening methods
	All evidence levels by clinical examination and was accepted for safety analysis inclusion	Non clinical studies Review High bias studies Expert opinions or commentary paper
Types of Participants	healthy adult blood transfusion donors who may or not may have their blood checked before with other screening tools besides the CMIA test.	

2.2. Assessment of study quality

All authors participated in summarizing and systematically assessing the evidence through the use of standard abstraction forms. The team will test the screening and abstraction forms on multiple articles before beginning the abstraction and review process. Screening and data collection forms may undergo revisions by the team. The results are presented in the evidence tables (Table 1).

2.3. Data synthesis

We did not conduct quantitative syntheses because of four factors. They are wide differences in how the condition being treated is operationally define across studies, large variety of interventions with rare replication of trials using the similar interventions, and disparate primary and secondary outcomes measure.

2.4. Data Extraction

Data extracted from the identified publication included: study design, locations, methods, participants, results, discussion, conclusions and comments. We used a table where each piece of information was written descriptively (Table 1).

3. Results

Our search identified 361 articles were identified, 184 were abstract and full-text screened which identified 3 articles that match the inclusion criteria and were included in our studies. The flowchart literature through the assessment process for the update of this review is shown in Flowchart 1.

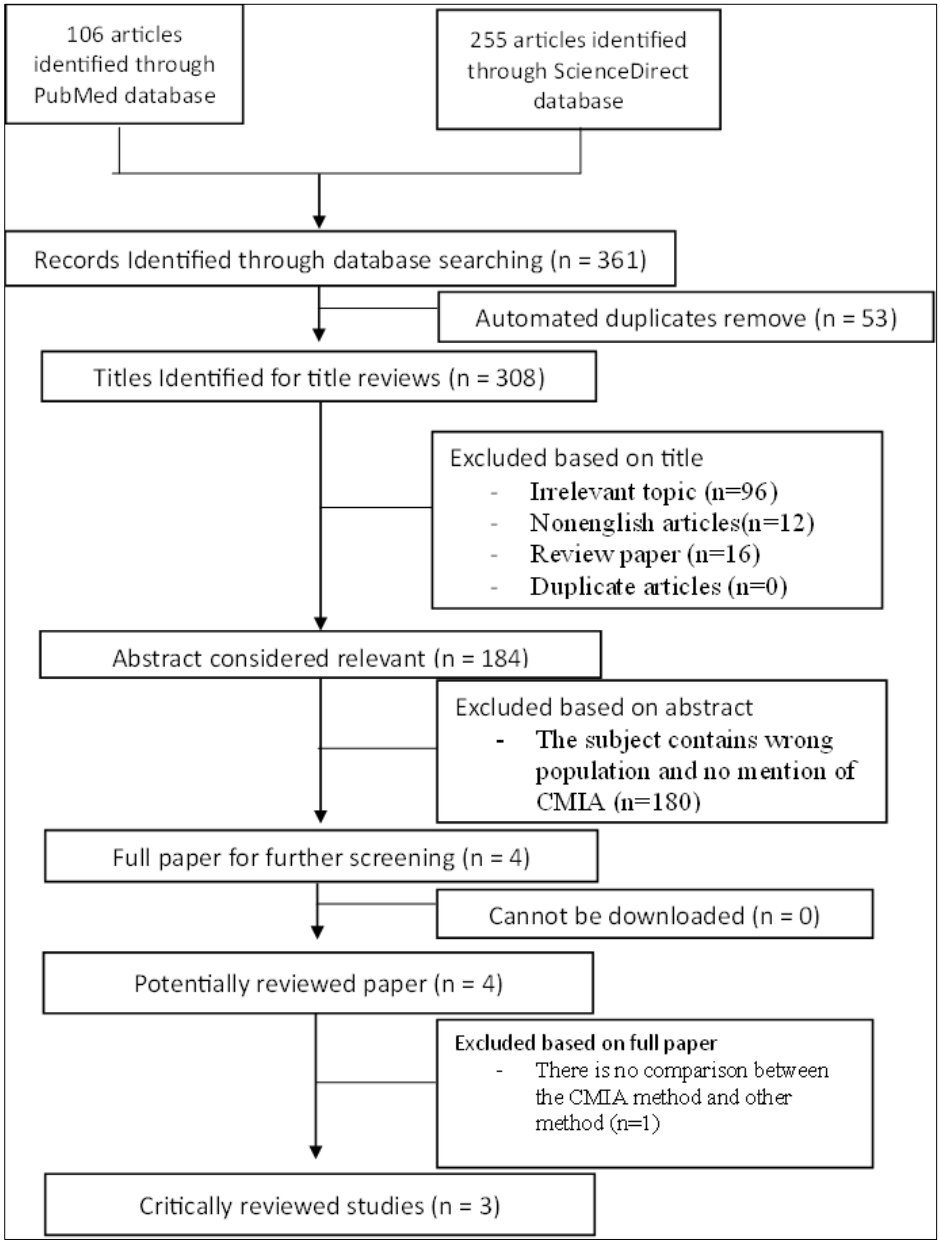

Figure 1 Study flow diagram of this review

Table 2 Characteristics and Outcomes of the included Articles

No.	Author	Locations	Methods	Participants	Results	Conclusions
1.	Cruz et al., 2023	HEMOPA foundation, Brazil	Restrospective, cross-sectional study	286.451 blood donor samples were screened for positive serology or molecular testing for HbsAg and anti-HBc	A total of 556 blood samples were HbsAg reactive, 3658 blood samples were anti-HBc reactive using serology testing 156 blood samples were both reactive for HbsAg and anti-HBc Of the 130/286,451 (0.05%) HBV NAT positive samples	The study proves that with the mentioned sensitivity and specificity of the Architect HbsAg and Architect anti-HBc using the

					found, 124 (0.04%) also tested positive for both HBsAg and Anti-HBc, 2 (0.0007%) tested positive for HBsAg alone, and 1 (0.0003%) tested positive for Anti-HBc alone. There were three (0.001%) reported NAT-only positive samples The distribution of the positive S/CO ratios in HBsAg ranged from 1.24 to 29,114.3. The distribution of S/CO ratios varied from 1.21 to 8912 for Anti-HBc positive readings. The optimal threshold for Architect HBsAg (CMIA serology testing method) was 404.15 (sensitivity = 96.83% and specificity = 89.21%), indicating a higher ability to distinguish blood donors with circulating HBV DNA.	CMIA method, we can most likely differentiate between viremic donors and non-viremic donors. However, we also need to perform HBV DNA testing which allows the identification of HBV DNA in people who test positive for Anti-HBc and negative for HBsAg.
2.	Viet et al., 2012	Quantri Medicine Centre, Vietnam	Cross-sectional	Healthy consenting adult age 18-55 years old living permanently in the area, A total of 1200 consenting participants were selected.	1200 blood samples were obtained and screened using EIA method for HbsAg, anti-HBC, anti-HCV. The results were then confirmed with the CMIA method. The EIA test method showed 11.4 percent of research samples (137/1200, 95% CI 9.6 - 13.2) tested positive for HBsAg, 51.7 percent (620/1200, 95% CI 48.8 - 54.5) tested positive for anti-HBc, and 9.5 percent (114/1200, 95% CI 7.9 - 11.3) tested positive for both HBsAg and anti-HBc. Among the serum samples, 42.2% (506/1200, 95% CI 39.4 - 45.0) were negative for HBsAg and positive for anti-HBc, whereas 1.9% (23/1200, 95% CI 1.2 -2.8) were positive for HBsAg and negative for anti-HBc. Two samples tested for anti-HCV were positive by CMIA and negative by EIA, this was speculated due to the different properties. The EIA were compared to the CMIA methods using the Kappa (κ) analysis with a result of 0.91 for HBsAg (95% CI: 0.83 -	This study concludes that by using the kappa analysis EIA method and CMIA method was high in agreement with a 98.7% sensitivity and 90.7% specificity for screening HbsAg. The study highlighted the importance of detecting HBV DNA since the prevalence of HbsAg negative anti-HBc positive samples were high. This was to be done to minimalize the miss screening for occult hepatitis b infection among blood donors.

					0.99), and 0.89 for anti-HBc (95% CI: 0.81 - 0.97). Values range 0.4-0.6 is "acceptable or moderate agreement", 0.6-0.8 is "good agreement" and 0.8-1 is "very good agreement	
3.	Chen et al., 2023	Shandong blood center, China	Experiment	Samples from 220,445 blood donors were taken from the Shandong Blood Centre. All donors gave their informed consent for blood donation prior to sample collection, and to confirm their eligibility, they performed a series of tests including a haemoglobin test, ALT quick test, ABO blood group test, HBsAg rapid test non-responsiveness test, and physical examination.	A total of 220.445 blood samples were tested for HBsAg, anti-TP, anti-HCV, and HIV/anti-HIV using ELISA reagents. Nucleic acid testing (NAT) was performed on any samples that tested positive for one reagent but negative for the other ELISA reagent. The study used the Abbott Architect i2000 chemiluminescence detection equipment to perform HBsAg neutralisation testing, HBsAg, anti-HBs, and anti-HBc CMIA testing on samples that had HBV DNA mixed-sample mode positive and single-sample mode positive results. Among the total samples, 67 samples were found to be ELISA negative but positive for HBV DNA. These samples were tested for hepatitis B using the Abbott CMIA five-item test at the National Clinical Laboratory (NCCL), and 25 of them were tested again using the Wantai CMIA confirmatory test. The results from both tests were consistent. The results of the study revealed that both the HBsAg and HBsAg neutralisation tests were negative in the CMIA testing for the 67 HBV DNA positive samples. Nonetheless, 12 samples (17.91%) tested negative for anti-HBc (window period infection, WP), while 55 samples (82.09%) tested positive for anti-HBc (OBI).	The study mentioned there were 67 blood samples that tested negative for hepatitis b serology marker by ELISA. Those samples were then tested with the Abbot CMIA method and some were tested positive for anti-HBc indicating an occult infection (OBI). This study suggested that anti-HBc was a critical serum marker for detecting OBI since it can be detected during asymptomatic infection and after recovery from HBV infection.

4. Discussion

This study looked at the use of the CMIA method as a screening tool for identifying an active HBV infection. We also looked for a comparison between screening tools such as the ELISA, rapid detection test (RDT) with the CMIA method. We hope that this study can provide a comprehensive understanding of the CMIA method and consider it as a screening tool for identifying HBV infection in blood donor centers.

There are not a lot of journals providing data regarding the use of the CMIA method as a diagnostic kit in blood donor centers, this was presumed to be because of the high cost and the need of experienced personnel to operate them, however, the CMIA method together with ECLIA have a higher specificity and sensitivity than other screening or diagnostic tools (5). In a 2023 study done by Cruz et al, showed that the Architect HbsAg and Architect anti-HBC using the CMIA method managed to identify a total of 4214 (1.47%) samples which were reactive for one or both serological markers, among them 556 (0.19%) were HBsAg reactive and 3658 were (1.28%) Anti-HBc reactive. A total of 156 samples (0.05%) were simultaneously reactive for HBsAg and Anti-HBc. Among the HBsAg positive samples, 126/ 265 (47.55%) were HBV NAT positive and 139/265 (52.45%) were HBV NAT negative. Among the Anti-HBc positive cases, 125/ 3062 (4.08%) were HBV NAT positive and 2937/3062 (95.92%) were HBV NAT negative. According to the study all cases with inconclusive results ($0.8 \leq S/CO < 1.2$) in serological screening for Anti-HBc or HBsAg were found to be HBV NAT negative. They also mentioned a strong correlation between high positive S/CO ratios with the HBV NAT's detection of circulating HBV DNA. In particular, grey zone results were not as indicative of viremia since HBV DNA was not detected at all in serologically inconclusive samples. Nevertheless, given that several HBV NAT-positive samples had low S/CO ratios during serological screening, this research emphasized the importance of improving blood transfusion safety by molecular screening (6).

In addition, with the use of molecular testing (ID-NAT), we could identify even the occult cases with minimal viral loads. The presence of anti-HBc further distinguishes OBI as seropositive or seronegative. In cases of seropositive OBI, the HBsAg level may turn negative years or decades after overt chronic HBV infection or shortly after acute hepatitis resolves. The window period (WP), also known as the seronegative OBI, is the period of time before HBsAg is found in blood and is defined solely by the presence of HBV DNA. One study mentioned it had managed to identify NAT yield cases (HBV DNA reactive) among HbsAg non-reactive samples. During the study time, it was discovered that the sample of 28,000 1304 donors was HBsAg non-reactive. A follow up screening using ID-NAT revealed that 25 samples wereHBV DNA reactive (NAT yield). Among these 25 NAT yields, 18 were solo NAT yields, 4 had NAT Co-yields, and 3 had NAT Co-Infection yields (one with HIV and two with HCV). The increasing number of occult infections showed the need for further NAT screening (7).

The study by Viet et al concerning Hepatitis B and Hepatitis C among potential blood donors in rural Vietnam mentioned the importance of detecting HbsAg and HBV DNA. The study collected approximately 1200 blood samples that were obtained and screened using the EIA method for HbsAg, anti-HBc, anti-HCV. The samples were then re-analyzed using CMIA method. Specimens with concentration values less than 0.05 IU/ml on the HBsAg CMIA test were deemed negative, and those with values more than or equal to 0.05 IU/ml were deemed positive. The ratio of signal to cut-off value (S/CO) serves as the foundation for the CMIA analysis of anti-HBc and anti-HCV. Positive values are classed as S/CO values more than 1.00, and negative values are classified as those less than 1.00. Agreement between the two methods were examined using Kappa analysis. Having kappa values more than 0.8, there was a very high degree of agreement between the results of the CMIA and the ELISA tests. However, the ELISA test's false-negative rates for anti-HBc and anti-HCV detection were greater than 5%. Up to 51% of HBsAg-negative anti-HBc-positive were reported in the study, which suggests that at least 10% of prospective blood donors in the research region might be carriers of latent hepatitis B infection (OBI) and which makes them a potential HBV transmitter. It is necessary to reevaluate the test quality utilised by the Vietnamese blood centres due to the significant prevalence of false-negative ELISA test results (8).

Another study suggests the use of two ELISA assay kits to make a ROC curve which was used to determine “gray zones” for detecting HbsAg. The study by Peng et al suggested the use of two ELISA kits the KHB assay and the InTec assay to determine the range of gray zone. They combined the cutoff value of each kit and determined that, for both the KHB and InTec HBsAg tests, the "gray-zones" were the S/CO values between 0.20 and 1.00. The result between the range values was considered to be borderline reactive. It is proved that the use of gray zone had a high sensitivity of 99.04% to detect HbsAg and no false positive samples were detected among the reactive samples detected by the two ELISA kits. The use of the gray zone maybe the solution to minimize false positive results made by the ELISA kit. The same study also mentioned the quantitative CMIA kit was reactive for 2.91% (3/103) of the samples whose S/CO ratios were below the lower "gray-zone" limits of the two qualitative assays, and these samples were subsequently verified as HBsAg positive. These three samples had low amounts of HBsAg (<0.10 IU/ml). In our research, the CMIA kit exceeded the ELISA kits in terms of sensitivity and specificity because it could distinguish true-positive HBsAg samples from those with HBsAg S/CO values lower than the ELISA kit "gray zones", additionally it could distinguish between true-positive HBsAg samples and those with uncommon serum HBV marker profiles and HBsAg S/CO values within the "gray zones” (9).

Chen et al mention in their study involving a total of 220.445 blood samples were tested for HBsAg, anti-TP, anti-HCV, and HIV/anti-HIV using ELISA reagents. Nucleic acid testing (NAT) was performed on any samples that tested positive on one serology test. Samples that were HBV DNA positive but ELISA negative were then re-analyze using Abbot CMIA.

During the time of the study, 67 samples were found to be HBV DNA positive but ELISA negative. These samples were tested for hepatitis B using the Abbott CMIA five-item test at the National Clinical Laboratory (NCCL), and 25 of them were tested again using the Wantai CMIA confirmatory test. The results from both tests were consistent. They then informed that both HBsAg and HBsAg neutralization tests were negative in CMIA testing for the 67 HBV DNA positive samples. Nonetheless, 12 samples (17.91%) tested negative for anti-HBc (window period infection, WP), while 55 samples (82.09%) tested positive for anti-HBc (OBI). To prevent transmission of OBI developed countries has started to screen for anti-HBc since it can be found both during the asymptomatic phase of the illness and during HBV infection recovery. According to this study, 77.36% of blood donors with positive HBV DNA tests but negative HBsAg tests also tested positive for anti-HBc. This indicates that up to 77.36% of HBV DNA-positive blood products can be clinically avoided by screening for anti-HBc (10).

There are not many studies that compare each screening tool in one study. One research in 2014 writes about the effectiveness of the four widely used detection techniques—ECLIA, GICA, ELISA, and CMIA—in identifying serum HBsAg. Three of the main methods used to detect serum HBsAg at this time are chemiluminescence, the enzyme-linked immunosorbent test (ELISA), and the golden immunochromatographic assay (GICA). Due to their many benefits, namely easy and practical use, quick detection, and low cost, ELISA and GICA have been extensively utilized for the qualitative screening of HBsAg. The advantage of the chemiluminescence techniques, like the chemiluminescent microparticle immunoassay (CMIA) and electrochemiluminescent immunoassay (ECLIA) was that they have high sensitivity and specificity and easy quantitative and automatic testing. The study first compares the imprecision of the four methods. When it came to the overall imprecision of the procedures evaluated by the guide of performance for precision and accuracy (EP15-A2), ELISA ranked lowest (14.9%), followed by CMIA (8.1%), and ECLIA (5.1%). The study done by Liu et al concluded that the ELISA method was more cost-effective, but the ECLIA, CMIA, and ELISA detection methods all proved suitable for qualitative testing while both ECLIA and CMIA were appropriate for the quantitative analysis (11).

Recently a study done in China evaluates and compares the detection of HBsAg using the ELISA technique and the CMIA technique. A total of 10,470 blood donor samples were tested simultaneously with ELISA and CMIA techniques. The wantai diagnostic kit was used for the CMIA and both the Beijing wantai and the Livzon or Xinchuang third-party ELISA reagents for the ELISA technique. The two ELSIA tools were then used to create a gray zone covering a range from 0 IU/mL to 1 IU/mL foe detecting HbsAg. The two ELISA reagents and CMIA showed excellent agreement throughout the study, with Kappa values greater than 0.82. In the 269 samples that showed double reactivity in the enzyme immunoassay (ELISA) tests, the CMIA demonstrated a 100% reactivity response rate. However, in the corresponding studies, CMIA yielded 14 and 6 false-positive results, with specificities of 99.73% and 99.89%. The two ELISA tests' detection limits were greatly surpassed by CMIA while analyzing samples in the grey zone serum plates. The two ELISA reagents had cutoffs of 0.1 IU/mL and 0.09 IU/mL, respectively, but the CMIA had a detection cutoff of 0.05 IU/mL. This study also mentioned that among 165 samples that tested positive for HBV DNA but negative for ELISA, CMIA identified 5 HBsAg-positive cases. This suggests that CMIA can identify HBsAg earlier, hence reducing the "window period". This is crucial since low viral load samples can benefit from missed detection due to the high sensitivity of CMIA. This study also provided data that when compared CMIA detected 296 positive samples, 15 subtypes, and 30 mutant HBsAg samples, all with a 100% sensitivity. Moreover, CMIA demonstrated a specificity of 99.81% among 10,411 negative blood donors, fulfilling the European Union criteria for blood screening reagent specificity at 99.50%. In conclusion, Wantai's CMIA is appropriate for screening blood donors due to its high sensitivity and specificity (12).

5. Conclusion

- The CMIA method has been used as a screening tool for detecting Hepatitis B infection in a few countries.
- The CMIA can identify HBsAg earlier, hence reducing the "window period". This is crucial since low viral load samples can benefit from missed detection due to the high sensitivity of CMIA.
- The CMIA demonstrated a specificity of 99.81% among 10,411 negative blood donors, fulfilling the European Union criteria for blood screening reagent.
- The Wantai's CMIA is appropriate for screening blood donors due to its high sensitivity and specificity.
- Anti-HBc was a critical serum marker for detecting OBI since it can be detected during asymptomatic infection and after recovery from HBV infection.
- Molecular testing such as NAT is still needed to make sure the presence of HBV DNA.

Compliance with ethical standards

Disclosure of conflict of interest

Author declares there is no conflict of interest during this research.

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