Asian Journal of Research in Biology

Volume 7, Issue 1, Page 13-24, 2024; Article no.AJRIB.11974

Screening Tests of Hepatitis B Virus Infection in the South of DR Congo: A Status Report

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/11974

Original Research Article

Received: 09/01/2024
Accepted: 13/03/2024
Published: 16/03/2024

ABSTRACT

In low-income countries with a high burden of hepatitis B and C viruses, it is important to develop inexpensive but effective strategies to diagnose and treat hepatitis. The aim of this study is to evaluate the sensitivity and specificity of the serum hepatitis B surface antigen (HbsAg) and that of the HBeAg envelope of the different tests used in the south of the DR Congo compared to the reference laboratory method.

Methods: By identifying tests in medical structures and collecting data on the principle of the test, antigen preparation, manufacturer, sensitivity, and specificity. These tests were further evaluated using samples previously evaluated by DNA PCR, sixty-five of which had non-detected results and twenty-one detected for the calculation of the evaluation parameters of a diagnostic test.

Results: 17 HBsAg tests were identified, 12 of which had a mention of antigenic preparation consisting of monoclonal and polyclonal AntiHBsAg antibodies and for which the Sensitivity for the test with was 93.65%, specificity of 99.35% for a PPV of 97.92% and an NPV of 97.97%; and for the 5 which did not mention it , the values are respectively: 90% for sensitivity, 92.87% for specificity, 87.09% for PPV and 92.87% NPV. And the evaluation of the HBeAg test compared to the HBV DNA PCR gave a sensitivity of 61.84%, a specificity of 78.55%, a PPV of 18.65% and an

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NPV of 96.28% for the tests including antigenic preparation mentioned and 57.14% of sensitivity, 76.689% specificity, 7.61% PPV and 097.55% NPV for tests which do not mention any.

Conclusion: The rapid tests used for HBsAg screening have low sensitivity compared to WHO recommendations and specificity within the standards. In low-income countries with a hepatitis B virus load, such as DR Congo, it is necessary to choose a screening test that is highly effective, easy to use, less expensive and gives rapid and accurate results. .

Keywords: Hepatitis B; HBsAg; screening; status report.

1. INTRODUCTION

"Hepatitis B virus, as a member of the Hepadnaviridae family, causes acute and chronic hepatitis in humans. Although there are approved antiviral drugs and vaccines, HBV infection remains a major global public health challenge, estimated to affect more than two hundred million people" [1]. "HBV virions are small and composed of relaxed, partially double-stranded, circular DNA of approximately 3.2 kb. The virus genome encodes four pre-genomic coding sequences (which serve as a template for genome synthesis), surface antigens (large, medium, and small), core (HBe and HBc) and x-transactivators (HBx)"[2] .

"Hepatitis B surface antigen (HBsAg) particles consist primarily of a 226 amino acid glycoprotein that carries B cell epitopes important for the induction of protective antibody responses in humans"[3]. "The region between residues 120 and 150 of the S protein has been clearly shown to represent the a-determinants common to all hepatitis B virus (HBV) isolates and is exposed on the surface of the HBV particle"[4] "Three outer envelope proteins, composed of hepatitis B surface antigen (HBsAg), these are three cocarboxyterminal glycoproteins that are encoded in the HBV S open reading frame by the alternating use of 3 translation initiation codons including large HBsAg [L-HBsAg], medium HBsAg [M-HBsAg] and small HBsAg [S-HBsAg] and lipid. SHBs are known to constitute the majority of HBsAg, while LHBs and MHBs form the minority and vary in subviral particles"[5].

"The three main serological markers used to determine HBV infection status are hepatitis B surface antigen (HBsAg), antibodies to hepatitis B surface antigen (anti-HBs) and antibodies against hepatitis B core antigen (anti-HBc). Serological markers change during the typical course of resolved acute infection and progression to chronic infection"[6]. "The new recommendations include screening for hepatitis B with three laboratory tests at least once during a lifetime for adults aged ≥18 years. The report also expands risk-based testing recommendations to include the following populations, activities, exposures, or conditions associated with increased risk of HBV infection" [7].

"Immunological tests to detect hepatitis B surface antigen (HBsAg) are commonly used for the diagnosis of HBV infection. The number of HBsAg particles is approximately 1,000 to 10,000 times greater than the number of complete DNA-containing viral particles, making HBsAg an extremely sensitive and useful marker for HBsAg infection". [8] "HBV. However, despite measuring HBsAg, there remains a residual risk of transmitted HBV infection mainly due to a relatively long pre-seroconversion period after HBV infection or occult HBV infection" [9, 10]. Therefore, there is a continued need to develop more sensitive HBsAg tests that can reduce the window period and detect occult HBV carriage.

"Additionally, HBV has been classified into 10 genotypes, designated A to J, based on >8% intergroup divergence in complete nucleotide sequences" [11]. "Indeed, a large number of amino acid substitutions have been found in the central region of amino acid residues 120 to 147 of HBsAg, and some amino acid substitutions affect antigenicity and immunogenicity" [12 ,13]. "Therefore, the sensitivity of immunoassays for HBsAg must be continuously improved to detect all genotypes and, at least, frequently observed escape mutants to reduce the risk of false negative results" [14].

"In view of the kinetics of serological markers of HBV infection which include HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc IgM and IgG. The identification of serological markers allows: to identify patients infected with HBV; elucidate the natural history of chronic hepatitis B (CHB); assess the clinical phases of infection; and to monitor antiviral treatment" [15].

It is known that there is a relationship between HBsAg, which appears in the serum in 1 to 10 weeks. And persists for more than 6 months implies chronic infection by HBV[16], with the transcription activity of ccDNA in the liver [17-19]and on the other hand between the serum titers of HBsAg are higher in patients with HBeAg-positive CHB[18-20] . indeed, HBeAg and anti-HBe were used to know infectivity and viral replication, but their use for this purpose was mainly replaced by HBV DNA testing [21] because although the Active viral replication is sustained in some patients with HBe seroconversion, certain mutations in the pre-core and central region inhibit or decrease HBeAg production[16].

It is within this framework that this work falls, which has set itself the following objectives:

- Identify the different hepatitis B screening tests in cities in southern DR Congo.
- Check their principles, their antigenic preparation, and their performance in terms of sensitivity and specificity according to the information provided by the manufacturers.
- Evaluate their performance against HBeAg, HBeAc and HBV DNA PCR

2. METHODOLOGY

This is a cross-sectional descriptive study conducted in the Democratic Republic of Congo precisely in the province of Haut-Katanga, the case of the city of Lubumbashi, Likasi and that of Likasi. It happened:

Through an interview which aimed to identify the distinct brands of rapid tests for screening for hepatitis B virus infection and conducted using a structured questionnaire composed of open and closed questions preformed in laboratories and medical structures from the city of Lubumbashi and Likasi. After conducting quality control on the consistency of the data collected, the encoding and analysis of the data were done using the Epi Info 7.3 software. We have, in this questionnaire collected: the brand of the test, its principle, its antigen preparation, sensitivity and specificity indicated by the manufacturer as well as the country of manufacture.

Then an evaluation of the HBV Ag tests according to the composition of their antigenic preparation using samples previously tested with the HBV DNA PCR, including 21 HBV DNA PCR detected and 65 HBV DNA PCR not detected in order to calculate Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value by group of tests according to the composition of their antigenic preparation was carried out for the qualification of the tests in relation to the WHO standards for rapid diagnostic tests. This set of samples was subjected at the same time to additional tests: HBeAg, HBe-Ac, HBc-Ac and HBs-Ac.

3. RESULTS AND DISCUSSION

The new global health sector strategy for hepatitis sets targets for the elimination of viral hepatitis as a public health threat by 2030 and sets outcome targets for the reduction of new infections and of mortality. These objectives are based on the establishment of screening and reliable diagnostic services which constitute a precursor to the implementation of effective treatment.

Key challenges to the current hepatitis testing response include lack of quality-assured and low-cost in vitro serological and virological diagnostics, limited testing facilities, inadequate data to guide hepatitis screening approaches, country-specific hepatitis, and lack of hepatitis screening guidelines in resource-limited settings.

After surveying 1439 laboratories, we identified seventeen tests of distinct brands which are marketed and in use in the south of the DR Congo and whose principle is that of immunochromatography for all the tests.

Table 1. Distribution of HBsAg tests according to antigenic preparation

Antigenic Preparation	NOT	%
Anti-HBs Ab	12	70.58
Unspecified	5	29.42
Total	17	100

We note that 70.58% of the HbsAg tests have a specific antigenic preparation which is the Anti-HBs Antibody and 20.42% of the tests have no antigenic preparation mentioned.

Indeed, in sub-Saharan Africa, many medical structures use rapid diagnostic immunochromo graphic tests (RDTs) for the diagnosis of infectious diseases, both at the point of care and in hospital laboratories, thanks to the many potential advantages they offer. in terms of transport and storage conditions at room temperature, not dependent on electrical power (other than for tests using plasma or serum requiring centrifugation), minimal user training, rapid turnaround time a result (usually within 15 minutes) [22].

The results of the undetected PCR DNA samples (N=65) and those detected (N=21) subjected to the HBsAg and HBeAg tests gave the following results: Of the 65 undetected HBV PCR DNA samples, the tests with HBs Ac antigen preparation gave a total of 5 positives compared to a total of 29 Negatives for tests without specific antigen preparation.

“The presence of HBsAg indicates HBV infection, whether acute or chronic, except when it may be transiently positive shortly after a dose of HepB vaccine”[23]. “the presence of HBsAg for at least 6 months defines chronic infection” [24]. “HBsAg detection remains the main diagnostic tool for HBV infection. HBsAg is produced in excess in HBV-infected hepatocytes and circulates in enormous quantities in serum, therefore, it is an extremely sensitive and specific biomarker of HBV infection”[25]. “However, the increased sensitivity of HBsAg tests can lead to false-positive results. Thus, in patients with HBsAg index values close to the threshold and with other inconsistent serological markers, verification of HBsAg positivity with a confirmatory test is recommended” [26]. In many laboratories, HBV testing is also feasible through rapid point-of-care (POC) testing. Point-of-care serology is available as a single test for HBsAg only or as detection of multiple serological markers. WHO recommends that an ideal POC test should meet the ASSURED criteria of being “affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users”. [27] “These tests are easy to perform and have a sensitivity greater than 90% and a specificity greater than 99.5%”[28].

Table 2. Distribution of HBsAg tests by antigen preparation and according to sample types Negative or positive for HBV DNA PCR

		DNA PCR Not Detected		DNA PCR detected	
		N=65		N=21	
Preparation	**Test HbsAg**	**Positive**	**Negative**	**Positive**	**Negative**
	1	0	65	19	2
	2	1	64	20	1
	3	1	64	19	2
	4	0	65	20	1
	5	0	65	20	1
	6	1	64	20	1
AntiHBs Ab	7	0	65	19	2
	8	1	64	20	1
	9	0	65	21	0
	10	1	64	20	1
	11	0	65	19	2
	12	0	65	19	2
	Total	5	775	236	16
	1	2	63	16	5
	2	1	64	17	4
Unspecified	3	3	62	15	6
	4	3	62	16	5
	5	3	62	17	4
	Total	12	313	81	24

Table 3. Distribution of anti-HVB protein antibodies according to the mention of the antigenic preparation

		PCR DNA HBV ND samples: N=65						PCR DNA DT samples: N=21					
		AcHBe		AcHBc		HBs Ab		AcHBe		HBc Ab		HBs Ab	
		N=65		N=65		N=65		N=21		N=21		N=21	
preparation	Test	P	NOT	P	NOT	P	NOT	P	NOT	P	NOT	P	NOT
	1	1	64	0	65	0	65	2	19	1	20	0	21
	2	2	63	4	61	0	65	1	20	2	19	0	21
	3	0	65	1	64	0	65	2	19	1	20	0	21
	4	2	63	0	65	1	64	0	21	0	21	0	21
	5	1	64	1	64	0	65	0	21	0	21	1	20
	6	0	65	0	65	0	65	2	19	0	21	0	21
AntiHBs Ab	7	1		2	63	1	64	0	21	0	21	0	21
	8	0	65	0	65	0	65	0	21	1	20	0	21
	9	3	62	3	62	0	65	1	20	0	21	0	21
	10	0	65	3	62	1	64	2	19	0	21	1	20
	11	1	64	2	63	1	64	3	18	1	20	1	20
	12	2	63	0	65	1	64	2	19	0	21	0	21
	Total	13	703	16	764	5	775	15	237	6	246	3	249
	1	1	64	0	65	1	64	1	20	1	20	1	20
	2	1	64	1	64	0	65	0	21	0	21	2	19
Unspecified	3	0	65	0	65	0	65	1	20	0	21	0	21
	4	2	63	0	65	0	65	1	20	0	21	1	20
	5	0	65	1	64	0	65	1	20	2	19	1	20
	Total	4	321	2	323	1	324	4	101	3	102	5	100

Screening for anti HBs Ag, anti Hbe Ag and anti HBc antibodies among HBV DNA PCR samples

In view of the CDC's 2023 Hepatitis B Virus Screening and Testing Recommendations, which requires that when universally testing for hepatitis B virus (HBV) by hepatitis B surface antigen hepatitis B (HBsAg), anti-HBsAg antibodies and total anti-HBcAg antibodies (total anti-HBc) are also sought [29] and constitute the three main serological markers used to determine HBV infection status. These serological markers change during the typical evolution of a resolved acute infection and the progression towards a chronic infection[a] as schematized by the Fig 1, which illustrates the typical serological evolution of an acute infection by the hepatitis B virus until recovery and the serological evolution typical of progression to chronic infection by the hepatitis B virus.[30].

“Indeed, the appearance of anti-HBs after a reduction in HBsAg indicates recovery from HBV infection. Among immunocompetent individuals never infected with HBV, anti-HBs at concentrations ≥10 muid/ml 1 to 2 months after completion of a HepB vaccine series indicate immunity”. [30] Total anti-HBc antibodies develop in all HBV infections, resolved or current, and generally persist for life. People whose immunity to HBV comes from a vaccine do not develop anti-HBc[31] and After identifying a person infected with HBV, testing for HBeAg, anti-HBe and HBV DNA can provide information on the level of viral replication and infectivity and help guide clinical management [30].

The search for HBeAg. for the unspecified tests of the antigen preparation, a total of thirteen positive results for HbsAg and six for HBeAg were found. The HBV DNA PCR samples detected a total of sixteen negative results for HBsAg and twenty-four for HbeAg.

3.1 Calculation of the Evaluation Parameters of a Diagnostic Test

The evaluation parameters of the HBs Ag test compared to the HBV DNA PCD are as follows, Sensitivity for the test with antigenic preparation Monoclonal and polyclonal anti-HBs Ag antibodies 93.65%, specificity 99.35% for a PPV of 97.92% and an NPV of 97.97% . for the test without precise antigen preparation the values are respectively: 90% for sensitivity, 92.87% for specificity, 87.09% for PPV and 92.87% NPV.

The results of the tests with mention of the antigen preparation showed high sensitivity, specificity, and precision for the hepatitis B virus,

which was comparable to those of a meta-analysis of thirty-three studies [32] which showed a pooled sensitivity and specificity of 90.0% (95% CI: 89.1-90.8) and 99.5% (95% CI: 99.4-99.5), respectively. Comparatively, in a meta-analysis performed in Korea, which is a high HBV burden country, the pooled sensitivity and specificity of serum HbsAg on RICT was 98.07% (95% CI: 97.67 -98.47%) and 99.56% (95% CI: 99.21-99.91%), respectively [33]. According to the current WHO procurement eligibility for HBsAg tests which requires that the rapid diagnostic tests can have a diagnostic sensitivity and specificity > 99% and > 98% respectively [34], it should be noted that the All the tests used in the south of DR Congo suffer from a problem of sensitivity rather than specificity.

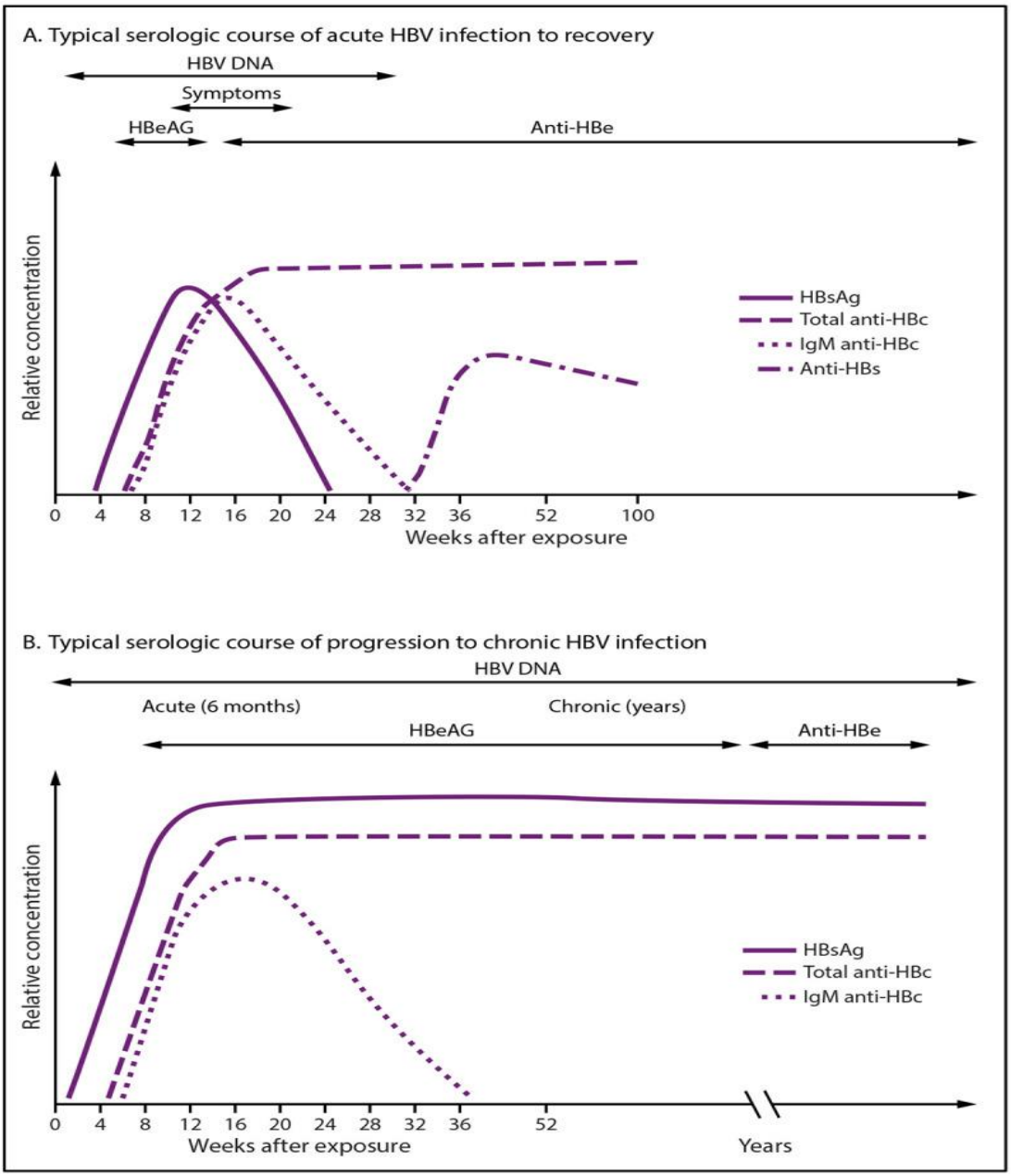

Fig. 1. Evolution of serological markers of viral infection with Hepatitis B Virus [30]

Abbreviations anti-HBc = Antibody Against the Main Hepatitis B Antigen; Anti-HBe = Antibody Against Hepatitis B e antigen; anti-HBs = Antibody against Hepatitis B Surface Antigen; HBeAg = hepatitis B e antigen; HBsAg = Hepatitis B Surface Antigen; HBV = Hepatitis B Virus; IgM = Immunoglobulin M

Table 4. Results of HBsAg and HBeAg tests on HBV DNA PCR samples detected (DT) and Not detected (ND)

		PCR DNA HBV ND samples: N= 65				HBV DT DNA PCR samples: N=21			
		HBsAg		HBeAg		HBeAg		HBsAg	
		N=65		N=65		N=21		N=21	
Preparation	**Test**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
	1	0	65	3	62	5	16	19	2
	2	1	64	2	63	6	15	20	1
	3	1	64	2	63	4	17	19	2
	4	0	65	3	62	5	16	20	1
	5	0	65	2	63	4	17	20	1
	6	1	64	3	62	4	17	20	1
AntiHBs Ab	7	0	65	3	62	3	18	19	2
	8	1	64	2	63	3	18	20	1
	9	0	65	3	62	3	18	21	0
	10	1	64	2	63	3	18	20	1
	11	0	65	2	63	4	17	19	2
	12	0	65	2	63	3	18	19	2
	Total	**5**	**775**	**29**	**751**	**47**	**205**	**236**	**16**
	1	2	63	1	64	2	19	16	5
	2	1	64	1	64	2	19	17	4
Unspecified	3	3	62	2	63	0	21	15	6
	4	3	62	1	64	1	20	16	5
	5	3	62	1	64	3	18	17	4
	Total	**12**	**313**	**6**	**319**	**8**	**97**	**81**	**24**

Table 5 A. HbsAg/ PCR DNA HBV test

HBsAg/HBV DNA PCR				
	Value	**95% CI**	**Value**	**95% CI**
Sensitivity	93.65	76.94% to 98.20%	90.00	78.95% to 98.20%
Specificity	99.35	93.91% to 99.79%	92.00	89.81% to 96.92%
Positive predictive value	97.92	80.14% to 98.45%	87.09	80.45% to 92.55%
Negative predictive value	97.97	92.79% to 99.12%	92.87	90.55% to 95.72%

Table 5 B. HbeAg / HBV DNA PCR test

HBsAg/HBV DNA PCR				
	Value	**95% CI**	**Value**	**95% CI**
Sensitivity	61.84	56.44% to 72.21%	57.14	36.45% to 68.25%
Specificity	78.55	63.92% to 81.97%	76.68	63.51% to 85.91%
Positive predictive value	18.65	4.54% to 28.55%	7.61	4.46% to 12.52%
Negative predictive value	96.28	91.79% to 99.62%	97.55	92.79% to 98.30%

The evaluation of the HBeAg test compared to the HBV DNA PCR gave a sensitivity of 61.84%, a specificity of 78.55%, a PPV of 18.65% and an NPV of 96.28% for the tests with the antigenic preparation mentioned and 57.14% sensitivity, 76.689% specificity, 7.61% PPV and 097.55% NPV for tests that did not mention the antigen preparation.

“Qualitative detection of HBsAg is the hallmark of HBV infection. Its presence for more than 6 months is pathognomonic of a chronic infection. HBeAg (Hepatitis B e-Antigen) or a viral protein made by the hepatitis B virus and released by infected liver cells into the blood, detects the amount of virus in the blood as a result of highly active viral replication .A positive HBeAg indicates important levels of virus in the blood and a person is considered contagious. A negative HBeAg indicates that there is truly little or no virus in the blood and a person is generally considered less contagious; this can sometimes

indicate that a person has a mutant hepatitis B virus"[25].

"A negative test result indicates that the virus may not be actively replicating in the liver. In general, a person is considered highly contagious when the test is positive, and less contagious when the test is negative. Loss of e-Antigen can occur naturally or as a result of drug treatment. Sometimes a negative test result can indicate the presence of a mutant hepatitis B virus. Thus, the absence of e-Antigen does not always mean that there is little or no active viral replication" [35].

"And studies conducted in patients positive for HBeAg, show a positive correlation between HBsAg titers, serum HBV DNA and liver cccDNA was observed"[36]. "On the other hand, this relationship has not been verified in HBeAg-negative cases of chronic HBV infection"[37]. "The lack of correlation could be a consequence of S gene mutations associated with HBeAg seroconversion, affecting HBsAg expression or secretion"[38].

At treatment initiation and HBeAg is used as a substitute for HBV DNA measurement to assess the risks of mother-to-child transmission[39-42] and given the high costs and difficulties related to access to HBV DNA testing in low-resource settings, WHO recommends HBeAg to triage treatment [43-44]; Therefore, the low accuracy of HBeAg POC testing is an urgent problem to be addressed.

As for the evaluation of the performance of the HBsAg and HBeAg tests compared to the HBV DNA PCR, our results show a variation in evaluation parameters; the sensitivity and specificity of HBsAg increase respectively from 93.65% to 90.00% for sensitivity and from 99.35% to 87.09% for specificity depending on whether the antigenic preparation is specified or not, the same goes for the search for HBeAg whose sensitivity values vary between 61.84% and 57.14% for 78.55% to 76.68% of the specificity. These results are similar to those evaluated in Malawi, where the sensitivity of HBeAg RDTs for detecting the HBV DNA treatment threshold of 20,000 IU/ml, as recommended in WHO treatment guidelines, has been demonstrated. ranged from 19.0% (95% CI 9.9, 31.4) to 44.8% (95% CI 31.7, 58.5). [45], A report from Senegal in West Africa also noted low sensitivity commercially available HBeAg RDTs [46]. But the sensitivity of HbeAg specificity improves with the increase of HBV DNA to the upper threshold of 200,000IU/ml to reach a sensitivity equal to 99.5% (95% CI: 91. 7-100) and a specificity of 62.2% (55.2-68.7). [47]

"Thus, in HBeAg positive patients, HBsAg was correlated with serum HBV DNA and low in HBeAg negative cases" [48]. "It appears that a cutoff value of 1500 IU/ml for serum HBsAg during treatment may be a predictor of seroconversion" [49] . "An effective HBsAg test is also urgently needed in settings to offer high accuracy (ideally >90%), excellent analytical sensitivity defined by the lowest detectable antigen concentration, the ability to detect mutants of HBV, provide results within minutes and have a longer shelf life at room temperature"[50].

"These POC tests target the surface antigenic region with the HBV determinant located between amino acid positions 99 and 160 of the HBsAg genome"[51]. Variation in HBV genotype resulting from amino acid changes within and outside the "a" determinant may affect HBsAg recognition and thus impact the specificity and sensitivity of rapid POC HBsAg.[52]. Genotype E, which is quite common in West Africa, the region most affected by HBV in Africa, has approximately 8% amino acid divergence from genotype A. However, an overview of the differences in HBsAg levels between HBeAg positive and negative patients, which appear to be affected by HBeAg status. HBV DNA levels were higher in HBeAg-positive patients, but HBsAg levels were higher in HBeAg-negative patients.[53]and there were clear differences in HBsAg levels depending on HBeAg status were observed.[54].

4. CONCLUSIONS

We observed that commercially available HBeAg rapid diagnostic tests have inadequate sensitivity for use in screening sites according to WHO standards. Our results highlight the importance of ensuring that diagnostic tests are evaluated in the environment where they will be used, to reflect local epidemiology, population, and viral genetic characteristics. There is an urgent need to develop HBeAg RDTs with improved sensitivity, suitable for use in sub-Saharan Africa and validated with locally prevalent HBV genotypes, to facilitate effective screening programs, treatment, and prevention of HBV.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/11974