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Optimizing Hepatitis B screening: A comparison of ELISA and rapid tests

Vero Hanitriniaina HARIJAONA VOLOLONTSOA [1,*], Anselme RAKOTOARIVELO [1], Andry RASAMINDRAKOTROKA [2], Ny Onja Niaina RAHARIJAONA [3] and Elga VERENAKO [3]

[1] *Ecole Doctorale interépistémique ONIFRA (University FJKM Ravelojaona) Madagascar.*
[2] *Faculté de Médecine, Université d'Antananarivo.*
[3] *Ecole Doctorale Gestion des Ressources Naturelles et Développement, Université d'Antananarivo.*

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Abstract

Hepatitis B remains a major global public health problem, particularly in areas of high endemicity such as Madagascar, where prevalence exceeds 8%. Diagnosis relies primarily on the ELISA immunoenzymatic technique, which is recognized for its reliability but is not widely available in resource-limited laboratories. Rapid diagnostic tests (RDTs) offer a practical alternative, but their performance in this context has yet to be evaluated.

This prospective descriptive and analytical study compared the performance of the Virucheck® rapid test and the Wantai Bio Pharm ELISA for the detection of hepatitis B virus surface antigen (HBsAg). The sample consisted of 74 serum samples from patients with sickle cell disease and subjects followed at the JRA University Hospital. The results showed a seroprevalence of 6.75% by TDR versus 9.45% by ELISA. The TDR had a sensitivity of 71.41% and a specificity of 100%, while the ELISA confirmed its superiority with sensitivities exceeding 95% and specificities close to 98%.

Beyond technical performance, the study highlighted a predominantly young population, reflecting the significance of mother-to-child transmission, as well as a notable seroprevalence among young adults linked to risky sexual behaviors. These findings underscore the urgency of early and targeted screening, combined with a strengthened vaccination strategy.

In conclusion, optimizing hepatitis B screening in Madagascar relies on the complementary use of rapid tests—suitable for community-based screening—and ELISA, which is essential for diagnostic confirmation and clinical follow-up. This approach represents a strategic pathway to reduce transmission, improve vaccination coverage, and strengthen the fight against hepatitis B in a context of high endemicity.

Keywords: Hepatitis B; Screening; Rapid Tests (RDTS); ELISA; Public health in Madagascar

1. Introduction

Hepatitis is defined as an inflammatory condition of the liver caused by infection, toxins or an immune response Gupta, S., et *al.* (1998). Among these, viral hepatitis represents a major global public health problem. The World Health Organisation (WHO) considers them to be the fourth global health priority, after HIV/AIDS, malaria and tuberculosis (WHO, 2016).

Infection with the hepatitis B virus (HBV) is of particular concern: more than two billion people have been exposed, of whom 296 to 350 million are chronic carriers, mainly in sub-Saharan Africa and East Asia (WHO, 2024). HBV is highly contagious, 50 to 100 times more so than HIV, and ranks among the top ten causes of death from infectious diseases. It

* Corresponding author: HARIJAONA VOLOLONTSOA Vero H

is also classified as the second leading human carcinogen after tobacco, due to its role in the development of cirrhosis and hepatocellular carcinoma (Hou et *al.*, 2005; Shepard et *al*., 2006).

In Madagascar, prevalence exceeds 8%, the threshold defined by the WHO to classify an area as having high endemicity (WHO, 2017). Despite this alarming situation, hepatitis B remains a neglected disease, even though it constitutes a genuine public health challenge. The virus is transmitted via various bodily fluids, including blood, sexual secretions, saliva and breast milk (Hou et *al*., 2005; Christophe Sifer et *al*., 2003).

In response to this spread, several immunological techniques have been developed to improve screening. In Madagascar, diagnosis relies primarily on the ELISA (Enzyme-Linked Immunosorbent Assay) method, which is recognised for its reliability but is considered costly and inaccessible in many laboratories. Rapid diagnostic tests (RDTs), on the other hand, offer a practical alternative for widening access to screening, particularly in resource-limited settings (Peeling et *al*., 2010; WHO, 2017).

A previous study conducted at the JRA University Hospital has already compared these two approaches for the detection of the HBV surface antigen (HBsAg). The question that arises is: what is the relative performance of each technique in the Malagasy context?

Thus, the aim of this study is to compare the ELISA method and the Virucheck® rapid test for the detection of HBsAg, in order to optimize hepatitis B screening strategies in Madagascar.

To achieve this objective, this study is structured in three parts: methodology, experimental results, and discussion accompanied by suggestions and future directions.

2. Methodology

The aim of this study was to compare the performance of two immunological techniques used to detect the hepatitis B virus surface antigen (HBsAg), a key marker of HBV infection. The evaluation focused on the Virucheck® rapid diagnostic test (RDT) and the ELISA method developed by Wantai Bio Pharm, recognised as the international gold standard for HBV screening.

The study was conducted at two leading centres in Madagascar: the Immunology Laboratory at the JRA University Hospital and the Laboratory for Training and Research in Medical Biology in Faravohitra, both recognised for their scientific excellence and their role in biomedical training and research.

2.1. Study population and sample size

The study population consisted of serum samples from sickle cell anaemia patients being treated at the JRA University Hospital and from patients seen at the JRA University Hospital's Immunology Laboratory. A total of 74 samples were included in the serum bank.

2.2. Criteria

- The inclusion criteria applied to patients who had given their verbal consent and for whom complete clinical data were available.
- The exclusion criteria applied to patients who had not given their consent or for whom clinical data were incomplete.
- Consequently, only consenting patients with complete clinical information were included in the study.

Variables studied

The main variables were:

- Serological result for hepatitis B by ELISA.
- Serological result for hepatitis B by TDR.

Pre-analytical phase

The pre-analytical phase comprised:

- Data collection: personal details, medical history (hospitalization, blood transfusion, jaundice, vaccination, surgery, antenatal screening), and risk behaviours (tattoos, piercings, intravenous drug use, unprotected sex).
- Sample transport: use of cool boxes, cryotubes and Eppendorf pipettes.
- Sample type: serum aliquots stored at −20 °C until analysis.

Analytical phase

The sera were thawed at room temperature and homogenized using a vortex mixer.

- Rapid diagnostic test (RDT): the Virucheck® HBsAg kit was used in accordance with the manufacturer's recommendations. This qualitative test is based on sandwich immunochromatography using monoclonal and polyclonal antibodies. Interpretation is visual:
    - Positive: two bands (T and C zones).
    - Negative: one band (C zone).
    - Invalid: abs
    - Invalid: positive control < 0.80.

Statistical analyses

The data were entered into Excel 2007 and analyzed using Epi Info. The parameters calculated were:

- Sensitivity = TP / (TP + FN) × 100
- Specificity = NP / (NP + FP) × 100
- Positive predictive value (PPV) = PP / (PP + FP) × 100
- Negative predictive value (NPV) = NP / (NP + FN) × 100
- Overall test value (OTV) = (PPV + NPV) / (PPV + NPV + FP + FN) × 100

3. Results

3.1. Epidemiological profile: age and sex of patients included

During this study, 74 patients were included, representing 74 serum samples. With a male-to-female sex ratio of 1.55, this study population consisted of 60.8% males (n = 45) and 39.18% females (n = 29), with males predominating. The age of the population ranged from 2 to 45 years, with a mean of 8.11 years and a median of 4 years. The 1–5 age group was the most represented with 22 patients (29.72%), followed by patients aged 6–10 years with a percentage of 28.37%. Consequently, the study population is young, consisting mainly of children. Those aged 21 to 45 years account for only 13.5% of this population.

Table 1 Distribution of the study population by sex and age

Characteristics		**Frequency (n=)**	**Percentage (%)**
Gender (n=74)	Male	45	60,8
	Female	29	39,18
AGE (n = 74)	] 0 – 5 years]	22	29,72
	] 5 – 10 years]	21	28,37
	] 10 – 15 years]	10	13,51
	] 15 – 20 years]	10	13,51
	] 20 – 25 years]	4	5,4
	] 25 – 30 years]	4	5,4

	] 30 – 35 years]	1	1,35
	] 35 – 40 years]	0	0
	] 40 – 45 years]	1	1,35

3.2. Data based on the presence of jaundice

Thus, 56% of the population exhibited jaundice, i.e. yellowing of the conjunctiva and mucous membranes caused by elevated levels of bilirubin produced by the liver. This is linked to abnormal liver function. The remaining 44% did not exhibit this condition.

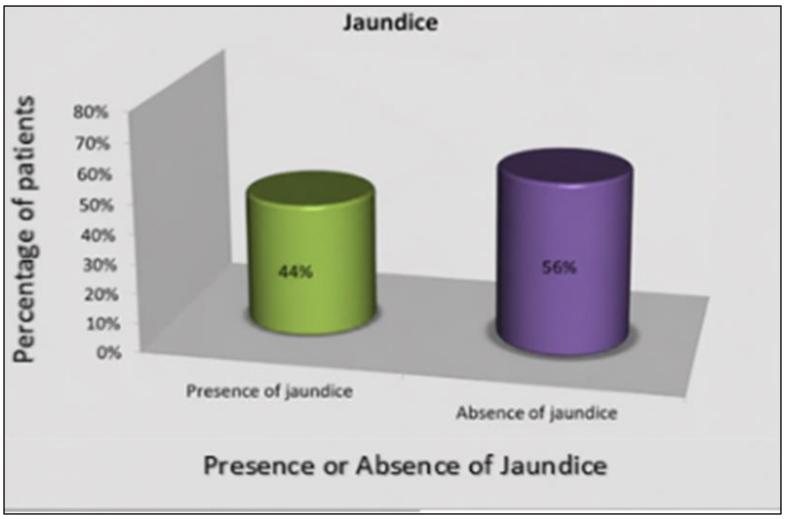

Figure 1 Breakdown of patients by presence of jaundice

3.3. Data on hepatitis B vaccination by gender

With regard to awareness of hepatitis B vaccination in Madagascar at present, 12.16% of the population have already been vaccinated, with 8.10% of males and 4.05% of females.

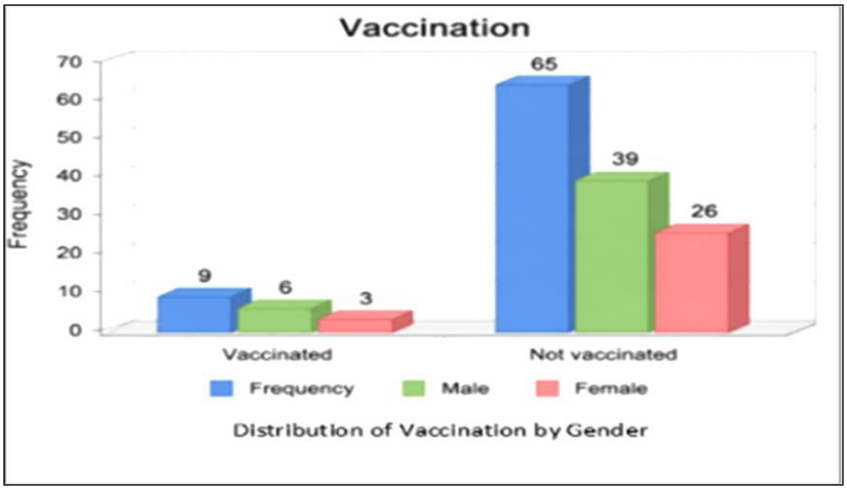

Figure 2 Vaccination rates by gender

3.4. Data on the frequency of blood transfusions and hospital admissions

According to the data, around three-quarters of the population have had a blood transfusion at some point, with 25.67% having undergone this procedure between 6 and 10 times, whilst 28.37% have had it between 2 and 5 times. Half of the

remaining population have had a blood transfusion only once, and the other half have never had one. The majority of the study population have never been hospitalised; however, 37.83% have been hospitalised at least once..

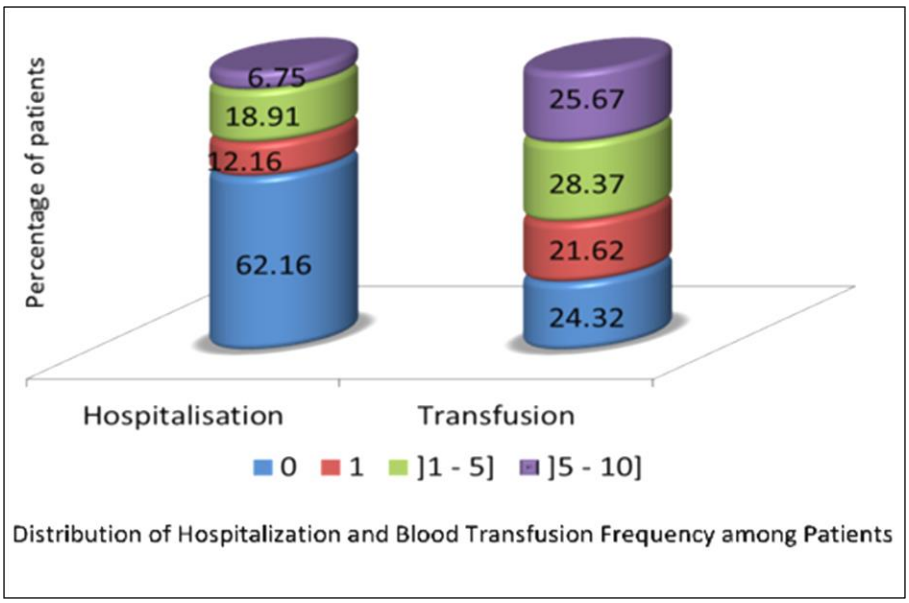

Figure 3 Frequency distribution of hospital admissions and blood transfusions among patients.

3.5. Data from hepatitis B screening

Of the 74 patients, only one 3-year-old child had undergone hepatitis B screening during their mother's pregnancy, despite this being part of the routine screening for pregnant women.

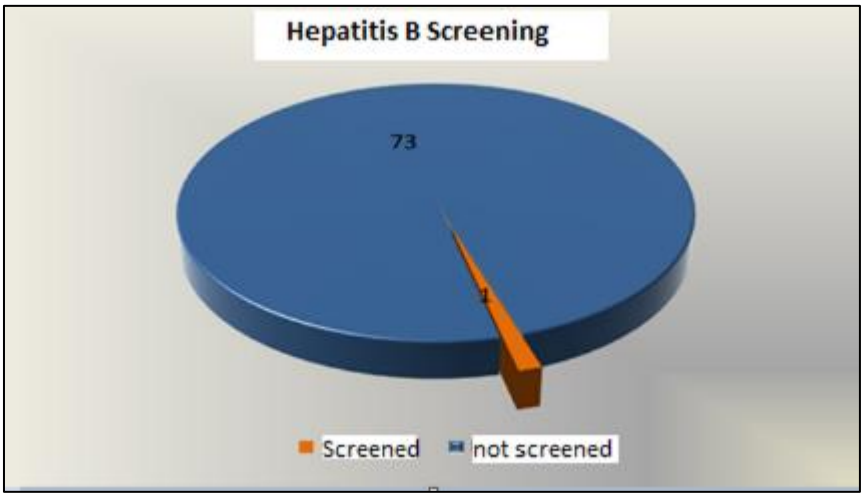

Figure 4 Distribution of hepatitis B screening during pregnancy

3.6. Data on other personal history

With regard to other personal history, 6.75% had engaged in unprotected sex, and 33.78% had piercings (the majority of whom were female). None of the participants had undergone surgery, had a tattoo, or engaged in intravenous drug use.

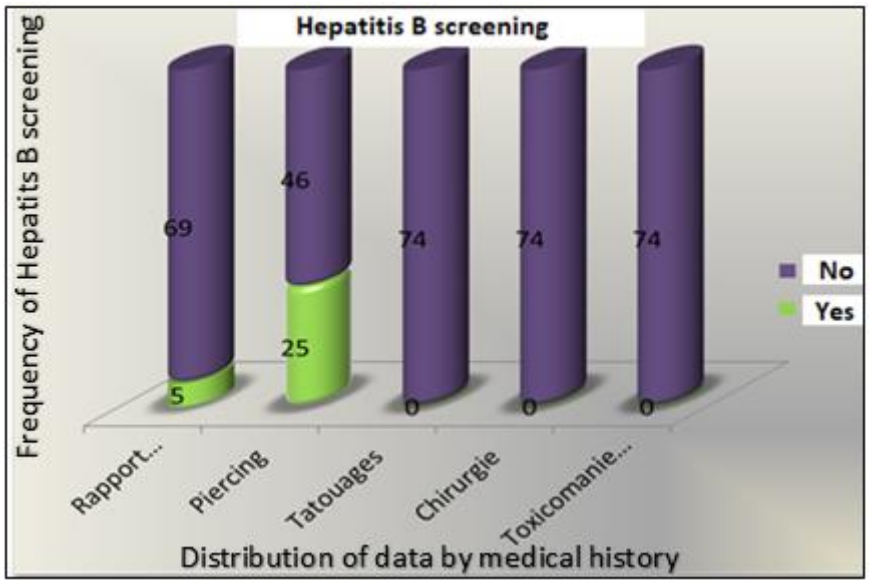

Figure 5 Breakdown of data by other medical history

3.7. Comparative study

Seventy-four (74) serum samples from patients with sickle cell disease and from individuals who visited the immunology laboratory at CHUJRA were first tested using the Virucheck® rapid test, and then using the Wantaï Bio-Pharm ELISA immunoenzymatic technique. The surface antigens present in the sera were assessed to diagnose hepatitis B in the samples. Both (2) techniques allow for a qualitative, rather than quantitative, measurement of HBsAg.

The two techniques differ significantly in their respective procedures, but prior to each test, the plasma samples are brought to room temperature, as are the reagents, and thoroughly mixed to prevent agglomeration at the bottom of the cryotubes. Each step is carried out meticulously to avoid any contamination.

A positive result in the rapid test is indicated by the appearance of two red bands, whereas a positive result in the ELISA is determined by calculation based on the COV and the optical density measured by the spectrophotometer.

Based on the analysis of the 74 serum samples, the results for the rapid test showed 5 positive and 69 negative cases, compared with 7 positive and 67 negative cases for the ELISA.

In principle, screening using the RDT is a first step in the detection of hepatitis B, but confirmation of the diagnosis must be carried out using a reference test such as the ELISA

Table 2 Distribution of HBsAg results from the two tests

	TDR	**ELISA**
Positive	5	7
Négative	69	67
Total	75	75

3.8. Seroprevalence of AgHbs using the TDR

The sera were first tested using the Virucheck® AgHbs rapid test, of which 5 were positive (6.75%), comprising 5.40% males and 1.35% females, whilst 93.24% were negative, comprising 55.40% males and 37.83% females.

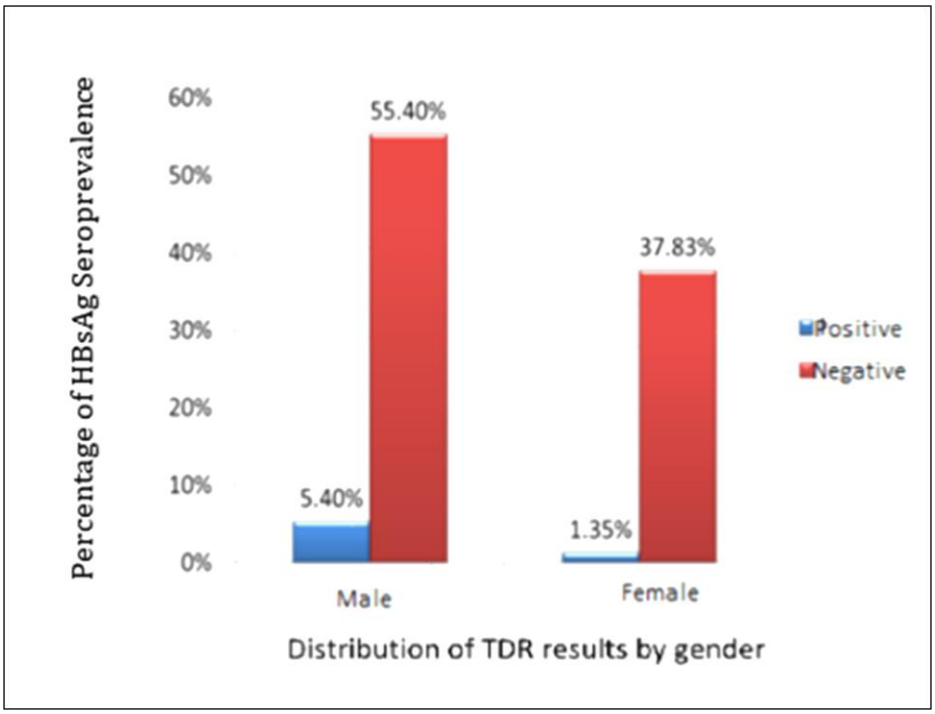

Figure 6 Distribution of results by gender, as a percentage, according to the TDR

3.9. Seroprevalence of HBsAg by ELISA

The seropositivity rate using the commercial ELISA kit from Wantaï Bio-Pharm is 9.45%, compared with 90.54% negative results, of which 6.75% were male and 2.70% were female.

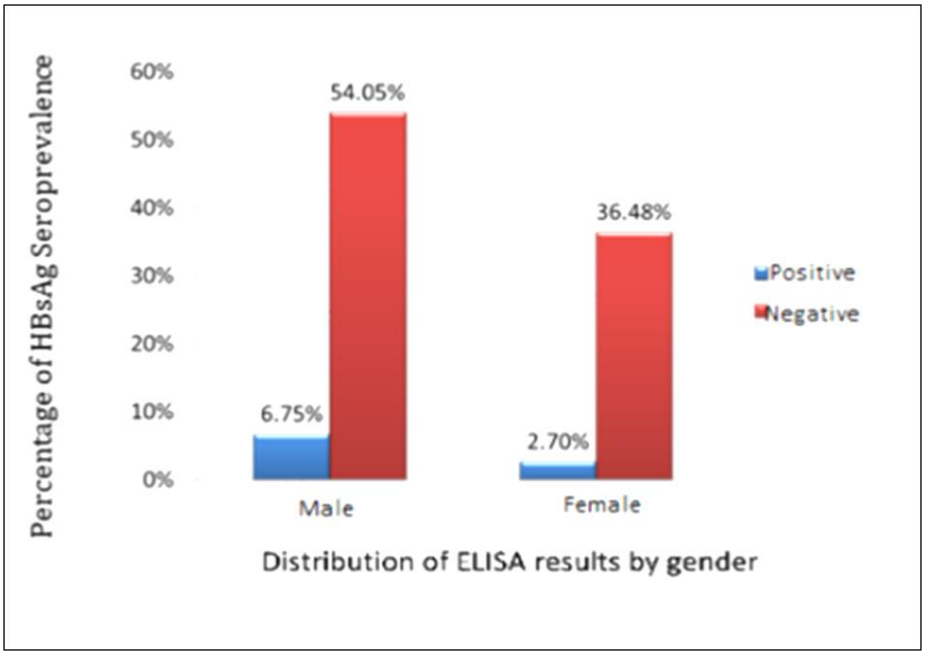

Figure 7 Distribution of ELISA results by percentage, by gender

3.10. Seroprevalence of HBsAg as determined by TDR and ELISA by age

Thus, HbsAg seropositivity by TDR is observed at a rate of 1.35% each for the 1–5, 16–20 and 25–30 age groups, whilst it is 2.7% among those aged 21–25.

For the ELISA, the 5–10 age group showed 2.7% positive sera, with the same figures as for the rapid test in the other age groups.

Table 3 Distribution of positive and negative sera by TDR and ELISA according to age

Age	**TDR**		**ELISA**	
	Positive	Negative	Positive	Negative
] 0 – 5 years]	1	21	1	21
] 5 – 10 years]	0	21	2	19
] 10 – 15 years]	0	10	0	10
] 15 – 20 years]	1	9	1	9
] 20 – 25 years]	2	2	2	2
] 25 – 30 years]	1	3	1	3
] 30 – 35 years	0	1	0	1
] 35 – 40 years]	0	0	0	0
] 40 – 45 years]	0	1	0	1

3.11. Seroprevalence of HBsAg as determined by TDR and ELISA according to medical history

Depending on medical history and HBsAg positivity, 3 out of 7 patients (confirmed as positive) presented with jaundice. One person out of the seven seropositive patients had been vaccinated against hepatitis B. As for blood transfusions, three people with HBsAg had received one, whilst four were already hospitalized. None of the seropositive individuals had undergone screening prior to the study. Two (2) of them had engaged in unprotected sexual intercourse. Only one person out of the seven has a piercing. For other risk factors such as intravenous drug use, surgery and tattoos, the percentages are zero.

Table 4 Distribution of risk factors according to HBsAg positivity (TDR vs ELISA).

	TDR	**ELISA**
	Among the 5 positives	Among the 7 positives
Presence of jaundice	3	3
Hepatitis B vaccination	0	1
Blood transfusion	1	3
Hospitalization	2	4
Hepatitis B screening during pregnancy	0	0
Unprotected sexual intercourse	2	2
Piercing	0	1

4. Discussion

4.1. Population characteristics and relevance of screening

In our study, 74 serum samples were analyzed, taken from individuals with sickle cell disease being treated at the CHUJRA and from patients diagnosed at the immunology laboratory. The mean age was 8.11 years, indicating a predominantly young population. The seroprevalence observed in the 1–10 age group is explained by mother-to-child transmission, a phenomenon extensively documented by Lavanchy et *al.* (2004) and confirmed by the WHO (2017), who note that perinatal transmission remains a major route of infection in areas of high endemicity.

Furthermore, among those aged 15 to 30 years, the seroprevalence corresponds to a sexually active population. This finding is consistent with the observations of Shepard et *al.* (2006) and Hou et *al.* (2005), who highlight the role of risky sexual behaviour in the spread of HBV. In our study, 28.5% of seropositive cases reported unprotected sex, which corroborates the findings of Ott et *al.* (2012) on the importance of behavioural factors in transmission.

Furthermore, a higher positivity rate was observed among men. This male predominance is confirmed by Migliani et *al.* (2000) in Madagascar, Traoré S et *al.* (2009) in Mali, and Antona et *al.* (2013) in France. Risky behaviours, particularly sexual ones, reinforce this observation. These data call for an in-depth analysis of immune factors and socio-cultural factors associated with being male.

These findings highlight the importance of early, targeted screening, particularly among children at risk of vertical transmission and among sexually active young adults. As Schweitzer et *al.* (2015) point out, the rapid identification of carriers not only helps to reduce transmission but also improves access to vaccination and clinical follow-up.

4.2. Clinical manifestations and modes of HBV transmission

In our study, three of the seven seropositive subjects presented with jaundice, a classic symptom of acute hepatitis. This clinical sign is widely recognized as an early indicator of liver damage, as described by Lok and McMahon (2009) and confirmed by Hou et *al.* (2005), who emphasise that jaundice remains a marker suggestive of acute HBV infection.

Blood transfusion was identified as a route of transmission in three patients, confirming the transfusion-related risks previously reported by Shepard et *al.* (2006) and Lavanchy et *al.* (2004). These authors emphasise the need to strengthen blood safety measures, particularly in countries with high endemicity where systematic donor screening remains a challenge.

Despite vaccination, one positive case was detected, which corresponds to the vaccine's partial efficacy (estimated at between 90 and 95%). This finding is consistent with the observations of the WHO (2017) and Schweitzer et *al.* (2015), who point out that, although highly effective, vaccination does not guarantee absolute protection, particularly in cases of an incomplete vaccination schedule or an insufficient immune response.

These results underscore the need to strengthen prevention measures, ensure rigorous post-vaccination monitoring, and promote targeted screening of at-risk populations. As highlighted by Ott et *al.* (2012), the combination of vaccination strategies and early screening is a key lever for reducing HBV transmission in endemic areas.

4.3. Performance and limitations of the rapid diagnostic test

The Virucheck® rapid test demonstrated a sensitivity of 71.41% and a specificity of 100%. These results are similar to those reported in India (Gupta et *al.*, 1998). Rapid tests offer major advantages for countries with high endemicity: speed (5–20 minutes), affordability, ease of transport and storage at room temperature. They are a first-line tool for mass screening, but remain limited to the detection of HBsAg and anti-HBs antibodies.

The Virucheck® rapid test demonstrated a sensitivity of 71.41% and a specificity of 100%. These results are consistent with those reported in India by Gupta et *al.* (1998), who observed a sensitivity of 77% and a specificity of 99%.

Furthermore, rapid tests offer major advantages for countries with high endemicity: speed of execution (5–20 minutes), affordability, ease of transport and storage at room temperature. These characteristics make them a first-line tool for mass screening, particularly in resource-limited settings. Several authors, such as Peeling et *al.* (2010) and Hou et *al.* (2017), emphasise that the accessibility and simplicity of rapid tests help to expand screening provision and improve access for vulnerable populations.

However, these tests have significant limitations. They are restricted to the detection of HBsAg and anti-HBs antibodies, and do not allow for the measurement of other serological markers that are essential for a comprehensive assessment of the infection. As noted by Hou et *al.* (2005) and Shepard et *al.* (2006), the lack of quantification and detection of specific antibodies limits their usefulness for clinical monitoring and diagnostic confirmation.

Ultimately, whilst rapid tests are a suitable solution for emergency situations and resource-limited settings, their use should be considered as a preliminary step. The use of reference techniques such as ELISA remains essential to confirm the diagnosis and ensure optimal management.

4.4. Superiority of the ELISA technique as a reference test

The ELISA immunoenzymatic assay (Wantaï Bio Pharm) is widely recognized as the reference method for screening for the hepatitis B virus (HBV). In Tulear, a study showed a seroprevalence of 69.7% with ELISA, compared with significantly lower rates obtained using other techniques such as immunodiffusion, electrophoresis or passive haemagglutination. Furthermore, the ELISA stands out for its ability to provide an objective and reliable quantification of serological markers. Several studies, notably those by Hou, Liu and Gu (2005) and Lok and McMahon (2009), confirm that this technique has sensitivities exceeding 95% and specificities close to 98%, making it an indispensable tool for accurate diagnosis and clinical monitoring.

Furthermore, ELISA not only detects HBsAg but also measures various HBV-related antibodies and antigens, thereby providing a more comprehensive view of the progression of the infection. As noted by Shepard et *al.* (2006) and the WHO (2017), this versatility is essential for distinguishing acute from chronic infections, assessing the post-vaccination immune response and monitoring seroconversions.

Ultimately, the results of our study, corroborated by the international literature, confirm the superiority of ELISA over rapid tests. Whilst the latter constitute a first-line solution in resource-limited settings, ELISA remains indispensable for ensuring a reliable diagnosis, appropriate management and better epidemiological surveillance.

4.5. Implications for public health strategies in Madagascar

The results of our study highlight the importance of incorporating rapid tests into community screening strategies, particularly in rural areas where access to healthcare facilities remains limited. As Peeling et *al.* (2010), rapid tests offer a pragmatic solution for expanding screening provision due to their speed, affordability and ease of use.

However, diagnostic confirmation via ELISA remains essential to ensure the reliability of results. Several studies, notably those by Hou et *al.* (2005) and Lok and McMahon (2009), emphasise the need to combine rapid tests with reference methods to ensure optimal patient care.

Furthermore, in a context of high endemicity such as Madagascar, the integration of these two approaches can improve access to screening, strengthen vaccination coverage and reduce transmission. The WHO (2017) recommends integrating systematic screening into national hepatitis B control programmes, with a particular focus on pregnant women and young adults. Furthermore, Schweitzer et *al.* (2015) point out that the early identification of chronic carriers is a key lever for reducing the global burden of the disease.

Ultimately, the implementation of a strategy combining rapid tests for mass screening and ELISA for diagnostic confirmation appears to be an effective approach to strengthening the fight against hepatitis B in Madagascar.

5. Conclusion

This study, conducted among 74 patients at the JRA University Hospital and the Laboratory for Training and Research in Medical Biology in Madagascar, compared the performance of the Virucheck® rapid test and the ELISA (enzyme-linked immunoassay) technique (Wantai Bio Pharm) in detecting the hepatitis B virus surface antigen (HBsAg). The results showed that, whilst rapid tests have the advantage of being accessible, quick and suitable for resource-limited settings, their sensitivity remains lower than that of ELISA (71.41% compared to over 95% for ELISA), although their specificity is excellent (100%). ELISA, meanwhile, confirms its superiority as the gold standard, offering diagnostic reliability and the ability to detect several serological markers essential for clinical monitoring.

Beyond technical performance, this study highlights worrying epidemiological realities: a predominantly young study population, reflecting the significance of mother-to-child vertical transmission, as well as a notable seroprevalence among young adults, linked to risky sexual behaviour. These findings are consistent with international observations and underscore the urgency of early, targeted screening, combined with a strengthened vaccination strategy.

From a public health perspective, the results call for a pragmatic integration of the two approaches:

- Rapid tests should be incorporated into community-based strategies to expand access to screening, particularly in rural and disadvantaged areas.
- ELISA should remain the test of choice for confirmation and follow-up, ensuring diagnostic accuracy and epidemiological surveillance.

Thus, optimizing hepatitis B screening in Madagascar relies on a balance between speed and reliability. This combination would not only reduce transmission but also improve vaccination coverage and access to treatment, in line with WHO recommendations. Ultimately, the implementation of a national strategy incorporating these two tools appears to be an essential step towards strengthening the fight against hepatitis B, reducing its disease burden and improving public health in a context of high endemicity.

Compliance with ethical standards

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We are pleased to submit our manuscript as an Original Research Article.

Disclosure of conflict of interest

The authors declare that they have no conflicts of interest related to this study.

Statement of Ethical approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Formal approval from an institutional ethics committee was not obtained. All participants were informed about the objectives of the study, participation was entirely voluntary, and all data were collected and analyzed anonymously to ensure the confidentiality of participants' personal information. Formal approval from an institutional ethics committee was not obtained.

Statement of informed consent

Oral informed consent was obtained from all participants prior to their inclusion in the study

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