EXPERT REVIEW OF MOLECULAR DIAGNOSTICS
2022, VOL. 22, NO. 5, 497–505
https://doi.org/10.1080/14737159.2022.2076595

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DIAGNOSTIC PROFILE



# Clinical utility of the 'Determine HBsAg' Point-of-Care Test for Diagnosis of Hepatitis B Surface Antigen in Africa

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**ABSTRACT**

**Introduction:** Chronic infection with hepatitis B virus (HBV) is a leading cause of morbidity and death, especially in sub-Saharan Africa (SSA), where approximately 60 million adults are infected. More than 90% of these patients are unaware of their HBV status.

**Areas covered:** Scaling-up of HBV screening programs in SSA are essential to increase diagnosis, linkage to care, and access to treatment, and will ultimately reduce HBV disease burden to achieve WHO hepatitis elimination targets. Such scale up will rely on inexpensive rapid point-of-care (POC) tests, especially in remote areas where gold standard serological assays are not routinely available. This review discusses the diagnostic performance and clinical utility of the Determine™ (Abbott, USA) hepatitis B surface Antigen (HBsAg) POC test for improving HBV screening in SSA, in light with others available HBsAg rapid tests.

**Expert Opinion:** The Determine™ HBsAg POC test has demonstrated relatively good diagnostic accuracy at the low cost, in the African field and laboratory and should be used for large scale mass screening of HBV infection in Africa.

**KEYWORDS**
Determine; HBsAg; Performance; Rapid diagnostic test; Africa

## Introduction

An estimated 248 million people are chronically infected with hepatitis B virus (HBV) worldwide, with the highest endemicity observed in sub-Saharan Africa (SSA), where approximately 10% of the adult population have chronic HBV infection [1,2]. In total, it is estimated that up to 60 million adults in SSA are living with chronic HBV infection [3]. More than 90% of these adults have never been tested and are not aware of their HBV status. Despite the availability of an effective vaccine and potent antiviral treatments, chronic HBV infection continues to be a major public health problem and a leading cause of morbidity and premature death. Chronic hepatitis B infection results in an estimated 887,000 deaths annually (3), mostly from cirrhosis and hepatocellular carcinoma (HCC). Up to 10% of HBV-related deaths occur in SSA [2]. In West Africa, up to 65% of HCC cases are attributable to chronic HBV infection [4] (Table 1).

For several decades, automated technologies have made it possible for clinical laboratories to perform many tests at a reasonable cost, usually taking several hours or days to obtain results and in some geographical areas these laboratories are not accessible. The need for faster results without the need for a clinical laboratory, especially in low-income countries, and the fact that some testing equipment has become portable, has led to an evolution in medical testing. Rapid tests cover many areas of medicine and are referred to by different names, such as 'near-patient testing,' 'remote testing'" 'satellite testing,' and 'rapid diagnostics.' Rapid tests include all tests that are performed at or near the patient and at the site where care or treatment is provided. Results are usually available within minutes, so they can be acted upon without delay.

The first step to diagnosis of HBV is the detection of hepatitis B surface protein (HBsAg) in the blood. Currently, multiple commercial point-of-care (POC) also known as rapid diagnostic tests (RDT) are available. They are qualitative lateral-flow chromatographic immunoassay, one step, easy to use, can analyze minute volume of specimens, of different types and provide visual results between 15 and 30 minutes. There are at least 4 World Health Organization (WHO) prequalified HBsAg POC kits and several more that have not been prequalified. A recent meta-analysis reported 33 brands, from 30 studies of RDT in 23,716 study participants. The studies were conducted in 23 countries during a period of 19 years. The objective of the meta-analysis has been to recommend a testing strategy for the WHO hepatitis guidelines 2017 [3].

As of 2020, four (4) HBsAg POCs including the Determine HBsAg 2 Abbott Laboratories, Abbott Park, IL, USA; Vikia HBsAg BioMerieux SA, Marcy-l'Etoile, France; SD BIOLINE HBsAg WB, Abbott, Standard Diagnostic Inc, Korea; and Standard Q HBsAg, SD Biosensor, India (12) were validated (prequalified criteria) and approved by WHO. These WHO prequalified rapid tests have shown high accuracy, sensitivity, and specificity in detecting HBsAg in community-outreach

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Table 1. World Health Organization (WHO) pre-qualified HBsAg rapid tests (as of March 2020)

| RDTs Name | Manufacturer | Characteristics | Pros | Cons | WHO prequalified |
|---|---|---|---|---|---|
| Determine HBsAg 2 (7D2942, 7D2943) | Abbott Laboratories, Abbott Park, IL, USA | 1. Is a visually read, qualitative immunochromatographic test.<br>2. It requires 50 µL of specimen.<br>3. Its lower limit of detection titer is from 1.0 to 0.52 IU/mL | 1. It can detect HBsAg in human serum, plasma, and whole blood. And it was validated for HBV genotypes<br>2. The results can be read from 15 to 30 minutes with small volume of sample.<br>3. It has high sensitivity and specificity. | 1. The intensity of the test band does not correlate with the titer of antigen in the specimen.<br>2. It was only validated against EDTA anticoagulants for whole blood, venous and capillary blood. | Yes |
| Vikia HBsAg® HBsAg (31124) | BioMerieux, France | 1. The test is intended for laboratory use by healthcare professionals only.<br>2. It is a qualitative detection of HBsAg in human serum, plasma, and whole blood<br>3. Is a strip impregnated with a goat polyclonal anti-Hbs antibody and a monoclonal anti-biotin antibody for test and control, respectively.<br>It requires 75 µL of specimen and LOD is 2 IU/mL | 1. It is easy to use and cost effective<br>2. It was validated against HBV sub-genotypes of adw2, (genotype A).<br>3. It was validated for several HBV genotypes.<br>4. It can be stored at room temperature of 25–40°C | 1. The test should not be used for postpartum and sample pools<br>2. It is designed for trained healthcare workers only.<br>3. Less sensitive than others. | Yes |
| SD BIOLINE HBsAg WB **(01FK10W)** | Standard Diagnostic Inc, Korea | 1. A rapid test designed for qualitative detection of HBsAg in human serum, plasma (heparin, EDTA, and sodium citrate), and venous whole blood.<br>2. It is intended for professional use only | 1. It can be used for various sample types.<br>2. It is easy to use and cost effective | 1. It is not suitable for early diagnosis or blood donation screening<br>2. It has not been validated for specimen collected from infants or children | Yes |
| Standard Q HBsAg | SD Biosensor, India | 1. . It is a rapid chromatographic immunoassay, designed to detect HBsAg in human serum, plasma and whole blood. | 1. It requires sample volume of (100 µL)<br>2. It can be stored in room temperature of 1–40°C<br>3. It is rapid and the results are obtained within 20 minutes. | 1. It is recommended to use alternative method obtain to confirmation of the results<br>2. It requires twice the volume of specimen when compared to Determine HBsAg2 POC<br>3. The lower limit of detection is not mentioned | Yes |
| Bioelisa HBsAg 3.0 | Biokit S.A | 1. It is a direct immunoenzyme sandwich technique. It is a microplate coated with guinea pig anti-bodies.<br>2. It captures antibody and goat anti-HBs antibodies with perioxides that serve as conjugate antibodies. | 4. It is more sensitive and specific with LOD of 0.100 HBsAg units/mL for ad and ay subtypes. Similarly, its sensitivity for genotype A subtype adw2 is at least 0.125 IU/mL<br>5. It has the ability to analyze large number of samples simultaneously. | 1. It requires cold chain storage of 2–8°C<br>2. Since it is ELISA, is more costly and requires trained personels and longer time to obtain results. | Yes |
| DS-EIA-HBsAg-0,01 (B-1254, B-1252 | RPC Diagnostics Systems | 1. It is a solid-phase, one-step sandwich enzyme immunoassay for qualitative detection and confirmation by neutralization. | 1. It is more sensitive and specific and can analyze 96 samples at the same time.<br>2. It has a high sensitivity and it is acheived by the incorporation of streptavidin-biotin technology. | 1. It is ELISA and requires cold chain storage.<br>2. It is expensive and requires trained laboratory personnel | Yes |
| Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 (9F80-01, 9F80-05 and 2G27-01) | Dia Sorin S.P.A UK Branch | 1. Is an enzyme immunoassay used for detection of HBsAg in human serum or plasma. Intended to screen blood donors of HBV infection. | 3. It is highly reliable and precise in detecting HBsAg for patient monitoring.<br>4. It can be used a first line of screening for HBsAg<br>5. It could be semi-automated or fully automated depending on available machine.<br>6. Validated for HBsAg of all known HBV genotypes A-H. | 1. The kit is recommended to be used at room temperature of 18–24°C.<br>2. The ELISA test is expensive and requires trained staff<br>3. It cannot be used in the field because it requires low temperature environment and automatic analyzer. | Yes |

screenings (32), health-facility-based screening and coinfected populations (2) especially from resource limited settings.

Beside the WHO prequalified HBsAg POCs, other newer brands of HBsAg POCs diagnostic tests are reported, which are equally effective for screening in the field. The DRW-HBsAg strip (Diagnostics for the Real-World Ltd., (CE-marked), the first response HBsAg card tests, Premier Medical Corporation (CE-marked), NaoSign ® HbsAg POC strip, Bioland and one step HBsAg test, General Biologicals Corporation Onsite HBSag combo rapid test (Abon Biopharm Hangzhou, China CTK Biotech); HBsAg Dipstick test (San Diego, USA); Genedia HBsAg (Cypress Diagnostics, Belgium); Hexagon HBsAg test (Green Cross Life Sciences Corp, Korea, Human, Wiesbaden, Germany), (32,35,36, 37, 38, 39,40), the list go on (Table 2).

Recently, multiplex POCs with high detection levels of HBsAg has been introduced. It is ideal for low-middle-resource countries with ability to detect many pathogens by one strip. Several are commercially available and are known as CE-marked such as (HBsAg/HCV/HIV/Syphilis Combo test, Euro Genomas; HBsAg and HCV Combo kit, Euro Genomas; Artron Detect 3 HIV/HCV/HBV Combo, Arton Laboratories; HIV, HBsAg and HCV Rapid test, Maternova Inc., Providence, RI, USA) on others. These assays have high accuracy; however, they are not prequalified by WHO (16, 29,74).

The basic utilization of HBsAg POCs is to determine seroprevalence in the general or specific populations in low-income countries. They have been applied massively in screening programs for chronic hepatitis B in communities and health-facility-based settings and have provided excellent merits to public health. For instance, in a community-based screening in the Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA), screen-and-treat program has helped to link nearly 1000 CHB carriers to care in the Gambia (2). It has been used in programs insert to engage hard to reach rural populations, people who inject drugs, sex workers and disadvantage groups or some ethnic groups (32). Of recent, the cost of these POCs have fallen dramatically making them more affordable for resource limited countries.

The affordability, cost-effectiveness, and simplicity of POCs have been the driving force of their usage. The cost of a lateral-flow-based HBsAg is generally lower than laboratory-based immunoassay, whereas most HBsAg are priced between US$ 0.2 to US $ 2.8 per test.

## HBV Testing

HBV diagnosis relies on serological detection of the hepatitis B surface antigen (HBsAg) by enzyme immunoassay (EIA) in whole blood, serum, or plasma. Access to HBsAg EIA testing is limited in SSA where sophisticated laboratories with reliable electric supply are scarce. Rapid HBsAg test kits, on the other hand, are cheap, easy to use, and do not require expensive equipment or highly trained personnel. POC kits have therefore replaced EIA testing in SSA.

HBsAg POC kits can scale up mass screening, improve safety of blood products in transfusion centers, accelerate clinical diagnosis in symptomatic patients with advance liver disease, and increase linkage to care and access to treatment HBV in patients living in SSA. Several HBsAg POC kits are available with many of these have acceptable analytical sensitivity to meet the acceptable performance requirements for WHO prequalification [5,6]. This review discusses the use of HBsAg POC tests and the clinical utility of the Determine™ HBsAg (Abbott, USA) POC tests for screening and diagnosis in Africa.

## HBsAg diagnosis – Unmet need

Most of the HBV-related deaths can potentially be averted with timely diagnosis and treatment. Antiviral treatment continuously suppresses HBV replication and can both regress liver fibrosis and prevent advanced liver disease. Expanded

Table 2. Diagnostic accuracy of determine validated against other POCs in the field and laboratory

| Year | POC Name | Manufacturer | Tests Site | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|
| 2020 | Determine™ HBsAg2 | Abbott | Laboratory | Plasma (98.6)<br>Whole blood (97.2)<br>Serum (97.9) | 99.8 (98.7–100)<br>99.5<br>100 | Avon et al. (18) |
| 2008 | Determine™ HBsAg | Abbott | Field | 8.5 (80.7–93.9) | 100.0 (99.5–100 | Parkin DM et al. (44) |
| 2008 | Determine™ HBsAg | Abbott | Field | 88.6 (75.4–96.2) | 100.0 (98.5–100) | J.J. Ott et al. (45) |
| 2012 | Vikia® HBsAg | Abbott | Field | 90.0 (79.5–96.2) | 99.8 (98.7–100) | Parkin DM et al. (44) |
| 2012 | Espline HBsAg | Abbott | Laboratory | 93.9 (89.1–97.1) | 94.7 (82.3–99.4) | Jemal A, et al. (46) |
| 1999 | Determine™ HBsAg | Abbott | Laboratory | 95.3 (90.5–98.1) | 93.3 (77.9–99.2) | Jemal A, et al. (46) |
| 1999 | Determine™ HBsAg | Abbott | Laboratory | 97.4 | 96.2 | Palmer et al. (48) |
| 2000 | Determine™ HBsAg | Abbott | Laboratory | 100 | 100 | Lien et al. (47) |
| 2008 | Determine™ HBsAg | Abbott | Laboratory | 97.8 | 100 | Randrianirina et al. (49) |
| 2008 | Determine™ HBsAg | Abbott | Laboratory | 55.9 | 69.4 | Nyirenda et al. (29) |
| 2008 | Determine™ HBsAg | Inverness | Laboratory | 77.4 | 100 | Lin et al. (22) |
| 2010 | Determine™ HBsAg | Inverness | Laboratory in the UK | 94.4 | 100 | Davies et al. (41) |
| 2010 | Determine™ HBsAg | Inverness | LocaL laboratory | 100 | 100 | Davies et al. (41) |
| 2012 | Determine™ HBsAg | Inverness | Local laboratory | 69.3 | 100 | Geretti et al. (19)<br>Guinea Malawi |
| 2013 | Determine™ HBsAg | Inverness | Laboratory in the UK | n/a | | Geretti et al. (19) |
| 2013 | Determine™ HBsAg | Abbottt | Local laboratory | 75 | 99.6 | Hoffmann et al. (42) |
| 2013 | Determine™ HBsAg | Inverness | Local laboratory | 93.6 | 100 | Bottero et al. (16) |
| 2013 | Determine™ HBsAg | Alere | Local laboratory | 96 | 100 | Franzeck et al. (43) |
| 2010 | Vikia | Biomerieux | Laboratory in the UK | 70.7 | 100 | Geretti et al. (19) |
| 2013 | Vikia | Biomerieux | Local | 96.5 | 99.9 | Bottero (16) |

Modified from Njai et al. 2015

access to testing for HBV is therefore critically important in order to reduce the burden of HBV-related morbidity and mortality and to achieve the ambitious targets for global elimination of viral hepatitis by 2030 to reduce hepatitis incidence by 90% and hepatitis deaths by 65%. Presently, less than 5% of people infected with HBV globally know their status [3]. Lack of awareness of HBV infection status deters linkage to care and access to treatment, thereby increasing risk of HBV-related morbidity and mortality. The WHO has therefore set baseline targets to diagnose 90% of HBV-infected individuals and 80% of chronic hepatitis B (CHB) patients who meet treatment eligibility criteria by 2030 [3].

Scaling up HBV testing is therefore crucial in increasing numbers of infected individuals aware of their HBV status, consequently increasing linkage to care and more effective response to the HBV epidemic [7,8]. Effective HBsAg testing is also urgently needed in antenatal settings to reduce HBV mother-to-child transmission (MTCT) in highly endemic regions [9,10].

In SSA, where there are an estimated 60 million adults with chronic HBV infection, HBV testing scale up using HBsAg EIAs testing is neither feasible nor affordable. HBsAg POC kits are therefore critical in filling this urgent unmet need in resource limited settings in SSA.

## Rapid POC HBsAg testing

The gold standard EIA tests for detecting HBsAg uses antibodies to HBsAg to capture antigen in a sample. Although very effective, EIA tests require high-quality laboratories with expensive equipment, cold storage of reagents, well-trained personnel, and constant electricity supply. Most of these logistics are often scarce in HBV endemic areas in SSA and Asia.

As a result, rapid POC diagnostic tests for HBsAg have become the first-line test for the diagnosis of chronic HBV infection in these settings. In contrast to EIAs, rapid HBsAg POC tests are user-friendly, inexpensive, avoid venipuncture by utilizing whole blood from finger prick (instead of plasma or serum), have a shorter turnaround time for results, and require less infrastructure, logistic, and training. POC tests therefore offer significant opportunities to scale up HBV testing, improve patient care, enhance workflow efficiency, and potentially yield financial benefits for hepatitis programs and patients in resource-limited settings. As a result, the WHO recommends the use of HBsAg POC tests to identify patients with chronic HBV infection [8].

HBsAg rapid POC tests work on the principles of immunochromatography, enzyme immunoassay, and chemiluminescence immunoassay. These POC tests targets the surface antigen region with determinant of HBV – the a-determine – located between amino acid positions 99 and 160 of the HBsAg genome. The clinical utility of a HBsAg POC kit depends on its overall performance both in the field and laboratory [11]. An ideal HBsAg POC kit must therefore offer high accuracy (ideally >90%) as well as excellent analytical sensitivity define by the lowest concentration of antigen detectable. In addition, the ability to detect HBV mutants, provide results within a few minutes and have a longer shelf-life at room temperature.

Since their introduction in the 1990s, numerous HBsAg rapid POC kits have been developed. The WHO Blood and Clinical Safety and Clinical Technology Department in 1998 initiated the prequalification of newly developed HBsAg POC tests. The main purpose of the initiation has been to provide objective assessments of commercially available assays for detection of HBsAg and hepatitis C virus (HCV) antibodies, as previously existed for human immunodeficiency syndrome (HIV) in 1988 [12,13].

This department perform the laboratory investigations, with the aim to supply those responsible for deciding which kits to use and provide comparative data for the available commercial HBsAg POC tests. The laboratory evaluation is performed in two phases, the first phase is performed in the WHO collaborating reference laboratory and it include limited panel of well-characterized samples. The second phase is conducted in two to four field (in the field) reference laboratories. This way, the two phases analysis will provide expanded information on type and origin of specimens in the evaluation panels. Thus, the data composes of analysis of various populations of different countries and laboratories to inform researchers and clinicians on the performance outcome of newly developed HBsAg POC in different regions [12]. Box 1 lists WHO prequalified HBsAg POC tests currently available in the market.

## Box 1. WHO prequalified HBsAg rapid POC tests (as of March 2020)

### *The Determine™ HBsAg test*

The Determine™ HBsAg POC test kit has been developed on the basis to meet the criteria for an ideal POC kit. It uses 50µL of plasma, serum, or whole blood from venipuncture or finger prick; provides results within 15 minutes; and has a shelf of 18 months at room temperature. The Determine™ HBsAg test strip is a visually read, qualitative immunoassay built on the principle of association of monoclonal and polyclonal antibodies specific to HBsAg (immunochromatography). It uses one-step qualitative, solid phase, two-site sandwich immunoassay for the detection of circulating hepatitis B viral antigen in blood and blood products [14,15]. These are designed to detect the antigenic determinant of HBV – the-a-determinant – located between amino acid positions 99 and 160 of the HBsAg.

The Determine™ was prequalified in 2001 by the WHO Blood Safety and Clinical Technology Department with a final sensitivity and specificity of 99.0 (94.5–100.0) and 99.4 (96.9–100.0 at 95 CL), respectively [13]. Several reports have since demonstrated the excellent diagnostic accuracy of the test in detecting HBsAg both in the field and laboratory in SSA, as well as its suitability for community-based large-scale screening in resource-limited settings [8–10]. A systematic review and meta-analysis of 27 studies showed that the Determine HBsAg POC kits has very high sensitivity (96.7%, CI 95.1–99.2) and specificity (99.6%, CI 97.0–99.9) in developing countries [14]. In this study assessing accuracy estimates of five HBsAg test brands (Determine, Hepacard, Genedia, BinaxNow and SD), the diagnostic odds ratios of Determine$^{TM}$

test performances were significantly higher in both studies done in developed and developing countries [14].

Further to this, our Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) group (www.prolifica.africa) assessed the diagnostic accuracy of three HBsAg POC tests (Determine™, Vikia and Espline (Fujirebio, Japan) to detect HBsAg using finger-prick whole-blood samples in the field and serum in the laboratory setting. Each result from POC tests was confirmed with the reference HBsAg ELISA test using AxSYM HBsAg ELISA (Abbott, USA) [11]. We found that the Determine POC kits had a sensitivity and a specificity of 88.5% and 100% in the field and 95.3% and 93.3% in the laboratory setting, respectively. The Determine POC kit had a higher specificity than Vikia in the field, although we did not find any evidence that Determine ™ performed better overall [11]. Similar studies comparing the Determine™ and other HBsAg rapid kits have shown a consistently higher sensitivity and specificity of the Determine ™ kit in both laboratory and field settings in SSA [15–17]. In developing countries, the rapid Determine ™ HBsAg POC test therefore provides a flexible, technically undemanding, and relatively inexpensive approach for HBV diagnosis.

Although similar levels of high specificity and sensitivity of the Determine ™ kit have also been reported in HIV-infected patients in Zambia [5], other studies in HIV- and HCV-infected patients in Africa showed lower sensitivity of this kit in these populations [3,17].

### 4.2 The Determine HBsAg2 kit

The new Determine ™ HBsAg test (product codes 7D2942 and 7D2943, Alere Medical Ltd) was launched in September 2019. This new POC kit has improved the limit of detection of 0.1IU/mL, overall sensitivity of 98.4% (95% CI 96.7–99.4%), and the ability to detect major HBsAg vaccine escape mutants. In 2019, WHO product performance evaluation of the HBsAg2 kit was done using clinically derived serum and plasma samples from European, African, Latin American, and Asian origin [6]. The evaluation concluded that the HBsAg2 kit had a specificity and sensitivity of 100 (95% CI 98.8–100) and 100 (95% CI 98.2–100), respectively [6].

In addition, Avellon and Co have validated the performance of the Determine™ HBsAg2 test kit in a multicentered study [18]. This study enrolled 365 subjects across five clinical sites in the UK and one clinical site in Spain. In the UK, participants were recruited in different clinics, including hepatology (for both adults and children), gastroenterology, digestive diseases' sexual health clinics.

The specificity and sensitivity of the results reported in this study for each sample types at reference cut-off of 0.05 IU/mL of the Determine HBsAg 2 test were high, making it an ideal tool for POC testing. It also has the potential for large-scale population-wide screening, which could result in scaling-up screening in resource-limited settings. Finally, the Determine™ test kit could be an improvement for the existing rapids tests since most of the reviewed rapid test do not meet the EU regulatory requirements of sensitivity and specificity [18]. In a nutshell, the results obtained from the total number of samples in each group when compared to architect HBsAg concentration specificity and sensitivity were higher than the existing HBsAg test kits [18].

## HBV Genotypes

To date, 8 HBV genotypes (A-H) and more than 30 subgenotypes have been described based on nucleotide differences. The genotypes show different geographical distribution and are related to disease progression, clinical progression, response to antiviral treatment, and prognosis. Genotypes A, D, and E are predominant in SSA, whilst B and C are most common in Southeast Asia. A-D and F genotypes are divided into different sub genotypes while E, G, and H have no subgenotypes [19,20].

Genotype A is commonly used as the reference virus type for the development of HBsAg diagnostic assays [21]. Variation in HBV genotype resulting from the amino acid changes within and outside the 'a' determinant may affect HBsAg recognition and thus impact on the specificity and sensitivity of rapid HBsAg POC [15]. Genotype E, which is very common in West Africa, the most affected region with HBV in Africa, shows approximately 8% of amino acid divergence from genotype A. Nonetheless, previous studies have indicated overall good performance of the Determine HBsAg test with HBV genotype E.

An independent field study performed in China and Guinea, two countries with high prevalence of chronic HBV infection of genotypes B and C (China) and E (Guinea), reported the sensitivity and specificity of both the Determine and DRW HBsAg test kit. The serotype and genotype sensitivity of the DRW and Determine HBsAg rapid test ranged from 0.15 to 0.8 IU/mL, respectively [22]. In addition, Scheiblauer H. and Co evaluated comparatively the performance of 70 HBsAg test kit from around the world to the gold standard for their clinical sensitivity, analytical sensitivity, sensitivity to HBV genotypes and HBV subtypes and specificity [23]. A reduced sensitivity for HBsAg with genetic diversity of HBV occurred with genotypes and subtypes D/*ayw3*,E/*ayw4*, F/*adw* and by S gene mutants. The specificity of the HBsAg assays was ≥99.5% in 57 test kits and 96.4–99.0% in the remaining test kits [23]. Chisenga, C.C et al. 2017, evaluated Determine HBsAg POCs field performance to ELISA and viral load levels among HIV/HBV coinfected Zambian patients. In this study, patients positive by the Determine HBsAg and ELISA were further analyzed for viral load levels and HBV genotypes. The POC test showed good sensitivity and high specificity of 87.9% and 99.7%, respectively. False negatives were also recorded and these negative samples when analyzed with qPCR had undetectable viral load and no evidence of significant liver disease. Even though samples positive by qPCR were genotyped, the author did not discuss the performance the Determine HBsAg kit for the different genotypes (26). The sensitivity of the kit is lower to samples analyzed in a monoinfection, which could be as a result of HIV coinfection which is reported to have impact on the performance of the test (42, 41, 43). With regard to the genotypes, the WHO validation data has reported high sensitivity of the assay for all the 15 HBsAg genotypes and serotypes (32).

## Limit of detection and sensitivity

The WHO recommends pre-screening of all new HBsAg rapid test strip with 1, International WHO HBsAg subtype *adw2*, genotype A reference Panel Catalogue number 03/262. All new POC tests should have limit of detection of 0.13IU/mL, sensitivity of 99% and specificity of 98% [6].

In our study [17], using the Determine HBsAg kit, the lowest HBsAg concentration level that showed reactivity with the Determine POC kit was 2.8IU/mL. False-negative results were reported in patients with very low HBsAg concentration, typically less than 1IU/mL. These patients with false-negative HBsAg tests were more likely to be older females, HBeAg negative, with low ALT and HBV DNA viral load levels. Importantly, in our study, none of the false-negative patients had significant liver disease. We can therefore assume that the impact of not being diagnosed with the HBsAg POC test should be minimal on the hepatic morbidity and mortality.

The new Determine HBsAg 2 kit has a reported limit of detection of 0.1IU/mL, making it the most sensitivity HBsAg POC kit currently in the market (22-WHO-Pg, 2019) [21,22].

## HIV coinfection

Chronic HBV infection is very common among HIV infected patients in SSA [24]. Despite international recommendations, HBV screening in HIV patients remains low (19). Rapid POC kits have therefore been introduced in several HIV treatment centers to fill this gap. Poor performance of HBsAg rapid POC kits have been reported in HIV patients compared to the general population. This could be a result of lower concentrations of circulating HBsAg levels and HBV DNA viral load in HIV-HBV coinfected patients, resulting in higher chances of false negative HBsAg test result with POC kit [7]. Moreover, antiretroviral therapy with reverse transcriptase inhibitors can modestly reduce HBsAg levels leading to false negative rapid test. Finally, HBV mutations resulting from Lamivudine (3TC), an antiviral drug used for HIV and HBV treatment, which is not recommended anymore due to drug resistance; Tenofovir and Entecavir, which have a much better drug resistance profile, are now the preferred antiviral drugs for treatment of chronic hepatitis B. Lamivudine (3TC) exposure can result in HBV surface genome variants undetectable by standard HBsAg POC kits [11]. Mutations in the 'a' antigenic determinant region of the HBsAg can cause conformational changes leading to decreased diagnostic accuracy [25,26]

Several studies have reported the performance of the Determine HBsAg test kit on HBV co-infection with HIV both in the laboratory and field settings in the low-resource countries. An evaluation study conducted in different regions of Zambia to determine the performance of the Determine HBsAg test kit to screen HBV coinfected HIV patients between 2013 and 2014 [27]. At urban HIV clinics in Lusaka, Zambia, the Determine HBsAg has a sensitivity of 88% among HBV-HIV-coinfected patients, and the detection was greater among those with higher HBV DNA levels [26,27]. Similarly, in 2017, meta-analysis, which included 9 studies with 7730 samples showed a pooled sensitivity of 90% and specificity of 99.1% using the Determine. However, the same study reported huge difference of sensitivity and specificity when the Determine was used to screen HBV HIV-infected patients [27–29].

The mechanism underlining the negative influence of lower sensitivity among HIV/HBV coinfection of Determine HBsAg POC is unclear; however, it may be attributed to cross reaction of HIV-reverse transcriptase enzyme inhibitors and HBV. Vicente N, et al [30]. reported higher rate of HBsAg loss among HIV/HBV coinfected populations (Vicente-Alcalde N, et al. 2020). The process has led to the increased number of occult hepatitis B (OBI) in early HIV cohorts in both treatment-naïve and treatment cohorts (Singh KP et al. 2017 [31]).

## The VIKIA® HBsAg

VIKIA® (BioMerieux, France) HBsAg is a qualitative test based on the association of monoclonal and polyclonal antibodies specific to HBsAg. The kit uses the principle of lateral immunochromatographic for the detection of circulating HBs antigen and is used for the detection of the main sub-types ad and ay in serum, plasma and whole blood (EDTA and Heparin) and whole blood by fingerpicks [5]. The WHO prequalification second International Standard for HBsAg, subtype adw2, genotype A set a cut-off for sensitivity to be less than or equal to 2.0 IU/mL for HBsAg test kit.

The clinical validation of VIKIA® of HBsAg test kit was performed to determine the sensitivity and specificity, where paired sample of 1800 plasma and venous whole blood were used. In addition, 1428 pair sample of whole blood included populations of blood donors, pregnant women [32], asymptomatic patients, positive HBsAg patients known to have chronic or acute hepatitis from West Africa, South America, and Asia [5]. The data for the analysis compared the sensitivity and specificity at 15 minutes and 30 minutes. The specificity for the first set at 15 minutes is 99.05% and the same relative sensitivity was reported. The data for venous whole blood reading at 30 minutes is 99.79% and for venous blood samples the sensitivity is 98.92%. Similar data was observed at 15 minutes [5,32].

Our group (PROLIFICA) found the sensitivity and specificity for VIKIA was 90.0% and 99.8%, respectively, in the field in SSA. Our data obtained from both field and laboratory show no significant difference between Vikia and Determine kits, with both having acceptable ranges of diagnostic accuracy and representing alternative to serology testing for the screening of HBV infection at the field level in sub-Saharan African [17]. In a recent meta-analysis [32] of three studies with 5,242 patients' samples, the Vikia HBsAg had a pool sensitivity and specificity of 82.5% [95% CI: 77.5, 86.7) and [95% CI: 99.8%, 100)], respectively. The data showed the Determine and VIKIA test had the highest pooled sensitivity and specificity [5,17,32].

### *4.4. SD Bioline HBsAg Test*

The SD Bioline HBsAg WB (Standard Diagnostic Inc, Korea) is an in vitro immunochromatography, one-step qualitative, solid phase, two-site sandwich immunoassay for detection of HBsAg in human plasma (heparin, EDTA, and sodium citrate) or venous whole blood and serum. The kit membrane is pre-

coated with anti-HBsAg antibodies on the test band region and anti-mouse anti-bodies on the control band region. When samples are injected into the test region, the sample reacts with the dye conjugate (mouse antiHBsAg anti-body colloidal gold conjugate). Sample reacts with antibodies on the membrane through capillary action and generates a band. The presence of double red bands indicates a positive result, and a single band of the control line indicates a negative result, whilst the absence of a red band at the control line indicates an invalid result [14,32]. The SD Bioline HBsAg rapid diagnostic test (RDT) is reported to have a 95% sensitivity and a 99% specificity [23,33]. Performance evaluation of 70 HBV surface antigen (HBsAg) assays from around the world by geographically diverse panel with an array of HBV genotypes and HBsAg subtypes [23,33,34].

## Conclusion

The Determine™ HBsAg POC kit has a demonstrated excellent performance in detecting HBsAg using capillary blood from finger-prick (in the field) or serum (in laboratory) in SSA. The kit has been shown to have an acceptable range of diagnostic accuracy, infrequent invalid results, and false-negative results predominantly in inactive chronic carriers at low risk of HBV-related complications. The Determine™ POC kit therefore should support the scale-up of large-scale HBV community screening programs in SSA to meet the WHO HBV elimination goals [35–51].

## Expert commentary (500-1000 words)

### *Unmet needs and key issues*

In most African countries, HBV screening is not free and patients must pay out-off pocket. This is a major obstacle to scale up screen-and-treat interventions for HBV in Africa. In addition, many African countries still do not have access to Determine™ HBsAg POC kits, HBV screening still relies on HBsAg serology, and therefore large-scale screening is not performed. Some populations remain neglected such as pregnant women, prisoners, men who have sex with men, or people living in remote areas.

Moreover, many African countries do not have national hepatitis plans and therefore improving and scaling up HBV screening is not listed as health priority in many of those countries.

In Africa, people are mainly infected early in life with HBV either from mother-to-child (vertical transmission) or from child-to-child (horizontal transmission). Since the risk of chronic infection and liver complications is higher in childhood as compared to adulthood, it is critical to improve screening early in life. HBsAg POC kits can be used easily in babies and children and are a painless diagnostic tool, that should be used during childhood to identify infants who escaped the HBV vaccination program in Africa. Interventions such as systematic screening based on HBsAg finger-prick test at school entry as well as systematic antenatal screening in pregnant women in a similar manner is done for HIV and other infectious diseases.

The Determine rapid POC test offers a reliable, inexpensive, and feasible tool to scale up HBsAg screening in children, schools, health facilities, antenatal clinics, and remote locations in SSA. The sensitivity and specificity of the Determine kit has been validated in the field in Africa and false-negative results are mostly limited to inactive carriers with low HBV viremia and no active liver disease and chronic carriers with mutated surface agent (HBsAg), which may not be detected by POCs. Finally, the current POCs are unable to identify people with occult hepatitis B infection (OBI), which means surface antigen negative and detectable HBV DNA. OBI is highly prevalent in endemic countries. Our research group has also found that OBI is an independent risk factor of HCC in The Gambia (unpublished data) as suggested by other studies (33).

### *5.1. Five-year view*

The last decade has experienced great efforts from the international health agencies and ministries of health of many countries to tackle the HBV epidemic. However, additional efforts are needed. African countries need to develop stronger strategies to fight against hepatitis B. As part their national hepatitis plans, it seems reasonable to consider a systematic HBsAg rapid testing of the general population living in SSA. At least a free single test during adult life should be considered. Indeed, in contrast to HIV, the risk of chronic infection with HBV is very low in adulthood, therefore adults need to be tested only once in their life.

The use of rapid POC test for HBsAg represents a good opportunity to fill the current HBV screening gaps in SSA.

## Funding

This paper was not funded.

## Declaration of interest

A Ceesay is funded by an Islamic Development Bank PhD fellowship.

G Ndow is supported by fellowships from Gilead Sciences USA and the MRC Gambia at LSHTM West Africa Research Platform.

M Lemoine is the recipient of MRC UK and ANRS funding and received consultancy fees and research support from Gilead, US Company.

D Cohen is funded by the ANRS grant 12357 B97.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

## Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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