RESEARCH ARTICLE

Assessment of rapid diagnostic tests and fourth-generation Enzyme-Linked Immunosorbent Assays in the screening of Human Immunodeficiency and Hepatitis B virus infections among first-time blood donors in Libreville (Gabon)

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Abstract

Background: Blood transfusion is a pathway for the transmission of blood-borne pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV) from donors to recipients in most countries in sub-Saharan Africa, including Gabon. The study aimed to compare the performance of four rapid diagnostic tests (RDTs: Alere DETERMINE, BIOSYNEX Exacto Pro HIV, MEDIFF HIV 1&2, and BIOSYNEX IMMUNOQUICK HBsAg) with results of 4th-generation immunoenzymatic assays COBAS 6000 e601 and EVOLIS BioRad for the detection of HIV and hepatitis B surface antigen (HBsAg) in blood donors in Libreville, Gabon.

Methods: Reactive and nonreactive blood samples for HIV and HBsAg were selected using fourth-generation ELISA COBAS 6000 e601 and EVOLIS BioRad. The sensitivities of RDTs were calculated using Epi Info version 6.04dfr (CDC, Atlanta, USA).

Results: Sensitivities for the detection of HIV in blood donors were 90.9% for Alere DETERMINE, 81.8% for BIOSYNEX Exacto Pro HIV, and 81.8% for MEDIFF HIV 1&2 Serum/sang Total Cassette compared with COBAS 6000 e601. The sensitivity of Alere DETERMINE compared to the semi-automated ELISA Bio-Rad for HIV detection was 65.6%. The sensitivity of BIOSYNEX IMMUNOQUICK HBsAg compared to ELISA tests for the detection of HBsAg was 78.0%. The specificity of all RDTs for the detection of HIV and HBsAg was 100%.

Conclusion: Alere DETERMINE HIV-1/2, MEDIFF HIV 1&2 Serum/sang Total Cassette, BIOSYNEX Exacto Pro HIV, and BIOSYNEX IMMUNOQUICK HBsAg are not recommended for determining whether donors qualify to donate blood because of their low sensitivity for the detection of HIV antibodies and HBsAg in blood donors in Gabon.

KEYWORDS

hepatitis B surface antigen, human immunodeficiency virus, Libreville, rapid detection test, sensitivity

1 | INTRODUCTION

Blood transfusion is a therapeutic act that saves millions of lives every year worldwide. However, it is also a major source of transmission of pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV). In 2015, the World Health Organization (WHO) estimated that 37 million people were living with HIV/AIDS worldwide, of whom 25.6 million resided in Africa, the most affected continent by this pandemic.¹ Hepatitis B virus is responsible for about 600 000 deaths worldwide.² An estimated 2 billion people are infected with HBV and 248 million are chronic carriers;³ 15%-40% of whom develop hepatic complications such as cirrhosis and hepatocellular carcinoma.

Transfusion safety is ensured by the strict selection of donors at low risk of blood-borne pathogen transmission and by the use of highly sensitive diagnostic tests capable of detecting pathogens even during the window period. In low-income countries and remote areas of sub-Saharan Africa (SSA), rapid diagnostic tests (RDTs) are used to test blood donations in the absence of immunoenzymatic tests.⁴ Their use does not require qualified personnel, and they are cheaper and well-adapted to the qualification of donations in small transfusion units. However, the sensitivity of RDTs is crucial. RDTs with sensitivities >99% are recommended by WHO.⁵

Among the four infections diagnosed routinely in Gabon (HIV, HBV, HCV, and syphilis),⁶ HIV and HBV are the two major infections observed among blood donors, with seroprevalences of 3.11% for HIV and 7.28% for HBV.^{7,8} In the capital city, Libreville, blood transfusion is validated by the National Blood Transfusion Center (NBTC), which has automated or semi-automated 4th-generation immunoenzymatic assays (COBAS 6000 e601) EVOLIS

BioRad (BioRad, Marnes-La-Coquette, France) that ensure acceptable transfusion safety for the recipient. However, in regional health centers and hospitals, transfusion units generally use RDTs to assess blood donations. The choice of RDT is often based on cost and time required, and most RDTs available for the routine diagnosis of HIV and hepatitis B surface antigen (HBsAg) or blood donation qualification have not been evaluated. In a previous study, we showed that COBAS 6000 e601 and EVOLIS BioRad had the same ability to detect HBsAg in blood donors.⁹

The purpose of this study was to evaluate the sensitivity of Alere DETERMINE™ HIV-1/2; MEDIFF HIV 1&2 Serum/sang Total Cassette and BIOSYNEX Exacto® Pro HIV for the diagnosis of HIV; and BIOSYNEX IMMUNOQUICK® HBsAg for that of HBV in Libreville blood donors.

2 | MATERIALS AND METHODS

2.1 | Blood donors

A cross-sectional study of blood donors in Libreville was conducted from August to November 2017. All volunteer and family donors selected were apparently healthy after responding to a panel of questions including their medical history. Excluded donors were those who received a transfusion, individuals with jaundice or signs of hepatitis, pregnant women, and those who reported risky sexual behavior during the six weeks prior to blood donation. The age for inclusion in blood donation was set from 17 to 57 years and the body mass ≥ 50 kg. Donors consented to the study, and information on their health status was recorded using a questionnaire. Venous blood was collected in blood collection bags following standard procedures.

FIGURE 1 Diagram of selected samples for rapid diagnostic test evaluation

TABLE 1 Comparison of RDTs and COBAS 6000 e601 for the detection of HIV in blood donors

RDT	Reactive	Nonreactive	COBAS reactive	COBAS nonreactive
Alere DETERMINE	20	27	22	25
BIOSYNEX Exacto® Pro HIV	18	29	22	25
MEDIFF HIV 1&2 Serum/sang Total Cassette	18	29	22	25

RDT, rapid diagnostic test.

2.2 | Selection of samples and anti-HIV and anti-HBsAg serology

Of 2200 samples tested using the 4th-generation ELISA, 227 samples were selected for the evaluation of RDT following the selection in Figure 1. The detection of p24 antigen and anti-HIV-1&2 antibodies, and HBsAg was performed using the Genscreen ULTRA HIV Ag-Ab and MONOLISA HBsAg Ultra (Bio-Rad, Marnes-la-Coquette, France). Elecsys HIV combi PT and Elecsys HBsAg II (Roche Diagnostics, Mannheim, Germany) were used to screen HIV and HBsAg using The COBAS 6000 e601. All reactive samples were confirmed by a second test using a different method. RDTs, Alere DETERMINE (Abbott rapid diagnostics, USA), MEDIFF HIV 1&2 Serum/sang Total Cassette (MEDIFF, Aubagne, France), and BIOSYNEX Exacto® Pro HIV and BIOSYNEX IMMUNOQUICK® HBsAg (BIOSYNEX, Illkirch-Graffenstaden, France), were evaluated in selected samples as shown in Figure 1.

2.3 | Statistical analysis

The performances of RDTs in the detection of HIV and HBsAg were determined using EPI Info version 6.04dfr (CDC, Atlanta, GA, USA). Sensitivity, specificity, and their 95% confidence intervals are presented. The Cohen's kappa statistical test (<http://graphpad.com/quickcalcs/kappa/>) was used to compare the concordance of RDTs in the detection of serological markers. Results were considered significant for $P < 0.05$.

3 | RESULTS

3.1 | Sensitivity and specificity of RDTs in the detection of HIV

Of the 22 reactive samples for HIV based on COBAS 6000 e601 results, only 20 samples were reactive with Alere DETERMINE and

TABLE 2 Sensitivity and specificity of RDTs compared to COBAS 6000 e601 in the detection of HIV

RDT	Sensitivity (95% CI)	Specificity (95% CI)
Alere DETERMINE	90.9% (69.4-98.4)	100% (83.4-100)
BIOSYNEX Exacto® Pro HIV	81.8% (59.0-94.0)	100% (83.4-100)
MEDIFF HIV 1&2 Serum/sang Total Cassette	81.8% (59.0-94.0)	100% (83.4-100)

RDT, rapid diagnostic test; CI, confidence interval.

18 were reactive with each of BIOSYNEX Exacto® Pro HIV and MEDIFF HIV 1&2 Serum/sang Total Cassette RDTs. All 25 HIV non-reactive samples with COBAS 6000 e601 were also nonreactive with RDTs (Table 1).

The sensitivity of Alere DETERMINE compared to COBAS 6000 e601 for HIV detection was 90.9% (95% CI = 69.4-98.4). The sensitivity of BIOSYNEX Exacto® Pro HIV and MEDIFF HIV 1&2 Serum/sang Total Cassette compared to COBAS 6000 e601 was 81.8% (95% CI = 59.0-94.0). The specificity of all RDTs for the detection of HIV was 100% (95% CI = 83.4-100) (Table 2).

We also evaluated the Alere DETERMINE RDT for the detection of HIV from samples selected by the 4th-generation ELISA EVOLIS BioRad. Of the 32 HIV-reactive samples using EVOLIS BioRad, only 21 samples were reactive with Alere DETERMINE. In contrast, all nonreactive samples for HIV with EVOLIS BioRad were also nonreactive with Alere DETERMINE (Table 3). The sensitivity of the Alere DETERMINE test compared to EVOLIS BioRad for the detection of HIV was 65.6% (95% CI = 46.8-80.8) while its specificity was 100% (95% CI = 93.8-100).

3.2 | Comparison of Alere DETERMINE and BIOSYNEX Exacto® Pro HIV or MEDIFF HIV 1&2 Serum/sang Total Cassette for the detection of HIV

Alere DETERMINE and BIOSYNEX Exacto® Pro HIV or MEDIFF HIV 1&2 Serum/sang Total Cassette agreed on the detection of HIV for 18 reactive samples and 27 nonreactive samples. The observed and expected by chance agreements of 95.74% and 51.74% were found, which gave a very good agreement between RDTs for the detection of HIV with a kappa value of 0.912 (95% CI = 0.793-1.000; Table 4).

TABLE 3 Comparison of Alere DETERMINE and EVOLIS BioRad in the detection of HIV

Alere DETERMINE	EVOLIS BioRad		Total
	Reactive	Nonreactive	
Reactive	21	0	21
Nonreactive	11	74	85
Total	32	74	106

Sensitivity = 65.6% (95% CI = 46.8-80.8); specificity = 100% (95% CI = 93.8-100).

TABLE 4 Comparison of Alere DETERMINE and BIOSYNEX or MEDIFF in the detection of HIV

	Alere DETERMINE			Po%	Pe%	Kappa (95% CI)
	Reactive	Nonreactive	Total			
BIOSYNEX or MEDIFF						
Reactive	18	0	18	95.74	51.74	0.912 (0.793-1.000)
Nonreactive	2	27	29			
Total	20	27	47			

P_o, proportion of observed agreement; P_e, proportion of expected agreement; CI, confidence interval.

TABLE 5 Comparison of BIOSYNEX IMMUNOQUICK® HBsAg and 4th-generation ELISA (COBAS/EVOLIS BioRad) in the detection of HBV

BIOSYNEX IMMUNOQUICK® HBsAg	COBAS/EVOLIS BioRad		Total
	Reactive	Nonreactive	
Reactive	39	0	39
Nonreactive	11	24	35
Total	50	24	74

Sensitivity = 78.0% (95% CI = 63.7-88.0); specificity = 100% (95% CI = 82.8-100).

3.3 | Sensitivity of BIOSYNEX IMMUNOQUICK® HBsAg compared to 4th-generation ELISA (COBAS/EVOLIS BioRad) in the detection of HBV

The positive HBsAg samples were therefore selected with two ELISA tests. Of the 50 HBsAg-reactive samples with ELISA, the BIOSYNEX IMMUNOQUICK® HBsAg identified 39 reactive and 11 nonreactive samples (Table 5). All 24 nonreactive samples for HBsAg by ELISA were also found to be nonreactive with BIOSYNEX IMMUNOQUICK® HBsAg. The sensitivity of BIOSYNEX IMMUNOQUICK® HBsAg compared to the 4th-generation ELISA for the detection of HBsAg was 78% (95% CI = 63.7-88.0) while its specificity was 100% (95% CI = 82.8-100).

4 | DISCUSSION

Despite the measures taken to improve transfusion safety, a residual risk of transmission of blood-borne pathogens persists. This risk is higher in developing countries.¹⁰ Due to the high prevalence of HIV and HBV in Gabon, the risk of transmission of these pathogens by blood transfusion is even higher compared to other countries where transfusion technology is developed. We assessed the sensitivity of RDTs for HIV (Alere DETERMINE, BIOSYNEX Exacto® Pro HIV, and MEDIFF HIV 1&2 Serum/sang Total Cassette) and HBV detection (BIOSYNEX IMMUNOQUICK® HBsAg) in Libreville blood donors. The sensitivities of RDTs compared to COBAS 6000 e601 for HIV detection were 90.9% for Alere DETERMINE and 81.8% for BIOSYNEX Exacto® Pro HIV and MEDIFF HIV 1&2 Serum/sang Total Cassette RDTs while their specificity was 100%. We also

determined the sensitivity of the Alere DETERMINE rapid test for the detection of HIV in blood donors with EVOLIS BioRad as reference. Alere DETERMINE had a sensitivity of 65.6% and a specificity of 100%. The sensitivities of Alere DETERMINE, BIOSYNEX Exacto® Pro HIV, and MEDIFF HIV 1&2 Serum/sang Total Cassette rapid tests for HIV detection were similar to that reported in South Africa, which ranged from 68.7% to 93.1%.¹¹

The RDT sensitivities found in this study are lower than those reported in Cameroon where the sensitivity of 12 rapid tests for HIV detection ranged between 93.7% and 100%.¹² The sensitivity of 90.9% for Alere DETERMINE obtained here is lower than that of 98.5% and 98.95% reported in previous studies in Nigeria and South Africa.^{13,14} The difference in RDT sensitivity could be explained by the choice of study populations. RDT sensitivities were evaluated on first-time blood donors who may have donated during seroconversion phase. A previous study in Gabon has shown low sensitivities of RDTs in HIV detection during seroconversion.¹⁵ These low sensitivities of RDTs could also be attributed to the influence of HIV variants, as reported in previous studies.^{12,16} According to the WHO, a high-performance RDT must have a sensitivity >99% for the detection of HIV.¹ A RDT with lower sensitivity than WHO standards increases the risk of obtaining false negatives, which represents a major challenge in blood transfusion.^{17,18} Alere DETERMINE and BIOSYNEX Exacto® Pro HIV or MEDIFF HIV 1&2 Serum/sang Total Cassette showed a very good concordance of 95.74% for the detection of HIV with a kappa coefficient of 0.912.

In this study, we evaluated the BIOSYNEX IMMUNOQUICK® HBsAg RDT for the detection of HBV. BIOSYNEX IMMUNOQUICK® HBsAg sensitivity was 78% (95% CI = 63.7-88.0). This sensitivity is higher than that of 51.5% reported in a previous study that assessed the sensitivity of rapid tests for the detection of HBsAg in blood transfusion centers in Africa.¹⁹ The sensitivity of 78% of BIOSYNEX IMMUNOQUICK® HBsAg determined in this study is lower than that of 98.5% reported for DETERMINE for the detection of HBsAg.²⁰ Relatively low antigen levels in blood donors may explain the low sensitivity of the BIOSYNEX IMMUNOQUICK® HBsAg test in the detection of HBsAg in the latter.⁵

All of the rapid diagnostic tests evaluated in this study for both HIV and HBsAg detection showed false negatives. The use of these RDTs in blood transfusion may be inappropriate for recipients because of their inability to reliably detect pathogens. A previous study has shown that the Genscreen ULTRA HIV Ag-Ab ELISA test (EVOLIS BioRad) used as

reference in this study for the detection of HIV could present false positives.²¹ Also, the Elecsys HIV combi PT kit used by COBAS 6000 e601 was developed to have a higher sensitivity allowing an early detection of HIV in the seroconversion phase.²² Pending the introduction of the viral genomic diagnosis in blood transfusion in Gabon, EVOLIS BioRad and Cobas 6000 e601 remain the most sensitive tests used in the diagnosis of HIV and HBsAg in blood donors. RDTs evaluated in this study should not be recommended first for blood donation qualification in Gabon, but they could be useful in avoiding blood shortages due to the 4th-generation ELISA reagents breakage in blood centers with limited financial resources. These RDTs could also be used in a predonation test strategy for HIV and HBsAg to reduce the qualifying cost of blood donations.^{23,24} Finally, in remote areas of SSA, in the absence of other diagnostic methods, these RDTs may limit the transmission of HIV and HBV by blood transfusion.⁴ Thus, improving blood screening tests is an important factor in reducing the residual risk of transfusion-transmitted infections.²⁵

5 | CONCLUSION

RDTs offer a new diagnostic opportunity in blood transfusion for low-income African countries. However, the genomic diversity of HIV and HBsAg is a serious limit to their uses. Therefore, they must be carefully evaluated and chosen for their use in blood transfusion in Gabon.

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AUTHOR'S CONTRIBUTIONS

CB and JMNN designed the study. SCME and CB did laboratory tests. CB and JMEM analyzed data. JMEM, CB, SCME, and BMN wrote the paper. CB, LEM, and BM corrected and approved the final version of the paper. All the authors read and approved the final version of the article.

ETHICS APPROVAL

This study was approved by the CNTS Ethics Committee.

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