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Comparison of electrochemiluminescence and ELISA methods in the detection of blood borne pathogens in Gabon

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ABSTRACT

Objective: To assess the performances of Cobas 6000 e601 and EVOLIS BioRad in the detection of HIV, HBV and HCV in blood donors in Libreville (Gabon).
Methods: A cross-sectional investigation was conducted in July 2017 in a total of 2 000 blood donors recruited at the National Blood transfusion Center, Libreville Gabon. Among them, 363 donors were selected to compare the performances of COBAS 6000 e601 (electro-chemiluminescence) and EVOLIS BioRad in detecting HIV, HBV and HCV using Cohen's kappa coefficient.
Results: Both methods yielded similar results for the detection of HIV and HBsAg. A very good agreement of 93.39% and an excellent agreement of 98.90% were obtained for the detection of HIV and HbsAg, with kappa values of 0.80 and 0.98, respectively. The observed agreement of 91.86% was found for the detection of HCV, which gave a fair agreement between the two methods with kappa = 0.33.
Conclusions: The two evaluation methods showed a similar performance in the detection of HIV, HBV. However, given the high rate of intra and inter-genotypes recombination known for HIV and HBV, more robust techniques of detection such as polymerase chain reaction should be used to prevent post-transfusion contaminations.

1. Introduction

Each year, blood transfusion saves millions of lives worldwide. However, in Sub-Saharan Africa, it remains a major route of transmission of infectious diseases such as human immunodeficiency virus (HIV), hepatitis B and C (HBV and HCV), which are major public health problems [1]. To effectively reduce the burden of transmission of these infectious agents, their diagnosis is essential [2]. Currently, many screening tests are available, including automated tests using electrochemiluminescence and enzyme-linked immunosorbent assay (ELISA) technologies, which are used in many countries [2]. The choice of a particular serological test in the diagnosis of an infection is based on the epidemiological data, and genetics of the infectious agents which could affect the assay limit [3,4].

In Gabon, the routine diagnosis of transfusion-transmitted infections (TTI) in blood donors at the National Blood Transfusion Center (NBTC) in Libreville, is carried out mainly by EVOLIS BioRad, a 4th generation ELISA test (dual detection of antigens and antibodies of infectious entities) adapted for blood transfusion [3,5].

The NBTC has recently acquired a new ITT's diagnostic tool using an electro-chemiluminescence technique (Cobas 6000 e601), whose performance has been evaluated previously [6,7].

This study sought to assess the performance of Cobas 6000 e601, using electro-chemiluminescence technology and EVOLIS BioRad (4th generation ELISA) in the detection of HIV,

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HBV and HCV in voluntary non remunerated blood donors in Libreville (Gabon) Central Africa.

2. Materials and methods

2.1. Donors' recruitment

A cross-sectional study on blood donors in Libreville was conducted in July 2017. All volunteer and family donors who are apparently healthy were selected after responding to a panel of questions including their medical history. The age for inclusion in blood donation cluster was set from 18 to 57 years old and the weight $\geq$ 50 kg. Donors were consented and information on their health status was recorded into a questionnaire. Excluded donors were those who received transfusion, individuals with jaundice or signs of hepatitis, pregnant women and those who had sexual risk behavior during the six weeks prior to blood donation.

2.2. Selection of samples and anti-HIV, anti-HBsAg and anti-HCV serology

Out of the 2 000 samples tested by the 4th generation ELISA, only 363 samples were screened for serological markers by COBAS 600 e601. The selection criteria for the samples are described in detail in Figure 1. The detection of p24 antigen, anti-HIV-1&2 antibodies, hepatitis B surface antigen (HBsAg), anti-HCV antibodies and antigens was performed using the Genscreen ULTRA HIV Ag-Ab, MONOLISA HBsAg Ultra and Monolisa HCV Ag-Ab ULTRA (Bio-Rad, Marnes-la- Coquette, France). ElecsysHIV combi PT, Elecsys HBsAg II and Elecsys Anti-HCV II (Roche Diagnostics, Germany) were used for the screening of HIV, HBsAg and HCV. All reactive samples were confirmed by a second test using a different method. All donors with undetermined serological status were convened for a second screening after 2–3 months.

2.3. Statistical analysis

Data was analyzed with the Statistical Package for Social Sciences software (SPSS version 20.0). The Cohen's Kappa statistical test (http:graphpad.com/quickcalcs/kappa/) was used to compare the concordance of the two diagnostic methods in the detection of serological markers.

2.4. Ethical considerations

This study was approved by the NBTC Ethics Committee. Informed consent was obtained from adults and parents or guardians of individuals under 18 years old before blood collection.

3. Results

3.1. Detection of HIV, HBV and HCV by ELISA and electro-chemiluminescence

Of the 363 donors selected for the detection of HIV, HBV and HCV, 75 donors were reactive to HIV by both methods. Two hundred and eighty-four (284) and 288 donors were non-reactive for HIV by ELISA and electro-chemiluminescence, respectively, while 4 donors were undetermined for HIV by ELISA (Table 1). Both methods displayed identical results for the detection of HBsAg with 160 reactive and 203 non-reactive donors.

Thirty six (36) and 11 donors were reactive to HCV detected by EVOLIS Bio-Rad and COBAS 6000 e601, respectively; 323 and 352 non-reactive donors were detected by EVOLIS Bio-Rad and COBAS 6000 e601, respectively, while 5 indeterminate samples were obtained only by ELISA (Table 1).

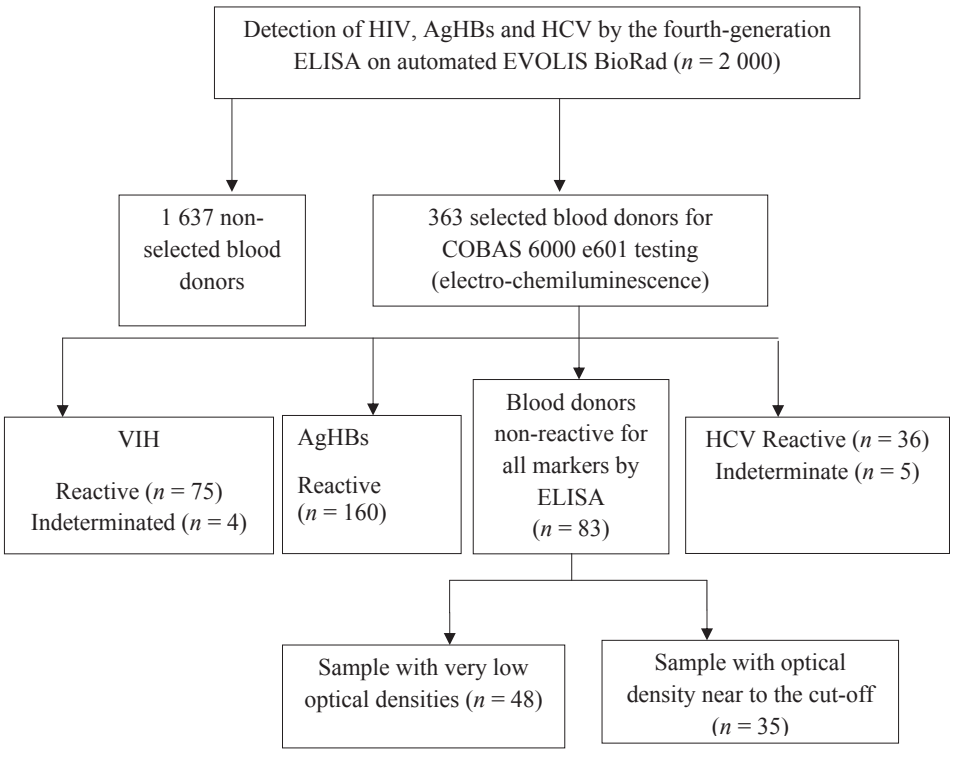

Figure 1. Diagram of selected samples tested with the two immunoassays.

Table 1
Repartition of HIV, AgHBs and HCV in selected blood donors after their testing with EVOLIS BioRad and Cobas 6000 e601.

Results	HIV		AgHBs		HCV	
	EVOLIS	COBAS	EVOLIS	COBAS	EVOLIS	COBAS
Reactive	75 (20.7)	75 (20.7)	160 (44.1)	160 (44.1)	36 (9.9)	11 (3.0)
Non-reactive	284 (78.2)	288 (79.3)	203 (55.9)	203 (55.9)	323 (89.0)	352 (79.0)
Indeterminate	4 (1.1)	0	–	–	5 (1.1)	0
Total	363 (100.0)	363 (100.0)	363 (100.0)	363 (100.0)	363 (100.0)	363 (100.0)

Note: EVOLIS: EVOLIS BioRad (4th-generation ELISA); COBAS: COBAS 6000 e601 (electro-chemiluminescence).

Table 2
Comparison of EVOLIS BioRad and Cobas 6000 e601 in the detection of HIV, AgHBs and HCV.

Results	HIV			AgHBs			HCV		
	Reactive	Non-reactive	Total	Reactive	Non-reactive	Total	Reactive	Non-reactive	Total
Reactive	65	10	75	158	2	160	8	24	32
Non-reactive	10	274	284	2	201	203	2	324	326
Indeterminate	0	4	4	–	–	–	1	4	5
Total	75	288	363	160	203	363	11	352	363

3.2. Comparison of COBAS 6000 e601 (electro-chemiluminescence) and EVOLIS BioRad (4th generation ELISA) for the detection of HIV, HBV and HCV

Of 363 donors tested for HIV, the two diagnostic methods detected 65 reactive and 274 non-reactive samples respectively. The observed and expected by chance agreements between the two methods were 93.39% and 66.34%, respectively, which showed a very good concordance with a kappa ± standard error (SE) value of (0.800 ± 0.038) (95% Confidence interval = 0.728–0.879) (Table 2).

With respect to the detection of HBsAg, the two screening methods were similar on 158 reactive and 201 non-reactive samples, which resulted in a 98.90% and 50.70% observed and expected by chance agreements, respectively, reaching an excellent agreement between the two methods with a kappa value of 0.980 ± 0.011 (95% Confidence interval = 0.956–0.999) (Table 2).

Electro-chemiluminescence and the 4th generation ELISA agreed on the detection of HCV for 8 reactive samples and 324 non-reactive samples. The observed and expected by chance agreements of 91.86% and 87.35% were found, which gave a fair agreement between the two methods for the detection of HCV with a kappa value of (0.330 ± 0.089) (95% Confidence interval = 0.158–0.492) (Table 2).

4. Discussion

Since the introduction of automated tests in the mid-1980s, screening for transmissible blood transfusion infections has progressively improved in terms of sensitivity and specificity [8]. At the NBTC in Libreville, two types of tests are used in the screening of ITTs: 4th generation ELISA and electro-chemiluminescence, whose performances have been evaluated in several previous studies [3,9–13].

In this study, EVOLIS BioRad and COBAS 6000 e601 showed a very good concordance for the detection of HIV and HBsAg. Indeed, for the detection of HIV, the proportion of agreement observed between the ELISA and electro-chemiluminescence was 93.39%, which gave a very good agreement between the two methods with a kappa coefficient of 0.80. A better agreement between the two methods with a kappa coefficient of 0.88 was reported in a previous study comparing ELISA with electro-chemiluminescence in HIV detection [9]. A match of 0.92 was obtained by comparing two automated tests using both electrochemiluminescence technologies (ARCHITECT and COBAS 600 e401) [14].

However, a discrepancy between the two tests was observed on 23 samples, which is likely due to the specific limitations of each kit in the detection of HIV. Indeed, the ElecsysHIV 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 [15].

Several previous studies have shown that these two kits were similar by detecting the same subtypes and different circulating recombinant forms of the M and O groups [16–18]. Another study suggested a higher sensitivity of the electro-chemiluminescence assay compared to the 4th generation ELISA because the former could detect more antigens [10]. In the present study, the electro-chemiluminescence test appeared to be more sensitive than the 4th generation ELISA, which eliminated the HIV indeterminacy found by the 4th generation ELISA and also detected several reactive samples that were non-reactive with EVOLIS BioRad.

The agreement between the two tests for the detection of HBsAg was excellent with a kappa coefficient of 0.98. These results are consistent with a previous study that found a 0.97 agreement between the 4th generation ELISA and the electro-chemiluminescence assays for HBsAg detection [2].

This excellent agreement between the 4th generation ELISA, electro-chemiluminescence, the Monolisa HBs Ag-Ab ULTRA and Elecsys HBsAg II kits could be explained by the fact that these assays have the same characteristics: same sensitivity (100%) and specificity (100%); and the same HBV genotypes (A, B, C, D, E, F and G) and subtypes (d, y, w1-w4, r and q) were detected.

In the case of HCV, the observed agreement was 91.96%, which gave a fair agreement between the two tests with a kappa coefficient of 0.33, and lower than the 97.78% reported in a previous study that evaluated a chemiluminescence assay

(ARCHITECT, Abbott) and a 4th generation ELISA test [19]. This fair agreement resulted in a significant discrepancy between COBAS 6000 e601 and EVOLIS BioRad for the detection of HCV. The kits used by COBAS 6000 e601 and EVOLIS BioRad, namely Elecsys Anti-HCV II and Monolisa HCV Ag-Ab ULTRA, did not detect the same HCV antigens and genotypes. The Elecsys Anti-HCV II kit detected capsid proteins, non-structural NS3 and NS4 proteins of genotypes 1 to 6, while Monolisa HCV Ag-Ab ULTRA detected capsid proteins and non-structural proteins NS3, NS4 and NS5 of genotypes 1 and 3.

In Gabon, HCV genotype 4 is predominant [20]. It is highly probable that the 4th generation ELISA has detected false positives because this immunoassay has been designed to detect HCV genotypes 1 and 3 which are minor in Central Africa [21], hence the high number of indeterminate samples was found in this test. The electro-chemiluminescence test, which detects HCV genotypes 1 to 6, did not produce indeterminate results.

According to Sommese *et al.* [14], this discrepancy is correlated with various factors, such as the large variation in the individual immune response to the different antigens used in each kit, and manufacturers using different vectors to clone recombinant antigens.

This study evaluated for the first time in blood donors in Libreville by the 4th generation ELISA (EVOLIS BIO-RAD) and electro-chemiluminescence (Cobas 6000 e601) tests in the detection of HIV, HBV and HCV.

The agreement between the two tests was very good for the detection of HIV and HBsAg while it was fair for HCV detection. The use of reference molecular tests would make it possible to determine the sensitivity and specificity of these two tests in the detection of HIV, HBsAg and HCV in blood donors in Gabon.

Conflict of interest statement

The authors declare no conflict of interest.

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