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Comparative study of two fourth-generation Hepatitis C screening ELISAs in blood transfusion in Ouagadougou: Monolisa™ HCV Ag-Ab ULTRA V2 (Bio-Rad) and Murex HCV Ag/Ab Combination (DiaSorin)

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A B S T R A C T

Safety of blood products is a global health priority. The aim of this study was to contribute to improve microbiological safety of blood products in Burkina Faso. Voluntary donors' blood sera collected at Ouagadougou Regional Blood Transfusion Center were tested by the Monolisa™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and Murex HCV Ag / Ab Combination assay (DiaSorin). The analytical performances of the two kits were compared. The sensitivity and specificity of Murex were evaluated using the Monolisa™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) as a reference. The differences were considered statistically significant for any value of $p < 0.05$. A total of 453 non-repetitive blood donations were tested in both kits. The detection rate of HCV markers by the Monolisa™ HCV Ag-Ab assay was 4.19% versus 5.52% for Murex HCV Ag / Ab. The difference in the detection of HCV markers between the two kits was not statistically significant ($p = 0.08$). The overall detection rate was 9.05%. The sensitivity was 15.8% and the specificity was 94.93%. The detection rate of HCV markers by the Murex HCV Ag / Ab Combination assay (DiaSorin) was higher than the Monolisa™ HCV Ag-Ab ULTRA V2 (Bio-Rad) one, but this difference was not statistically significant. However, the combination of these two kits in blood transfusion gave a higher detection rate (9.05%) and will provide more secure blood products.

Introduction

The hepatitis C virus (HCV) was identified in 1989, using a molecular cloning approach (Choo *et al.,* 1989). Globally, more than 150 million people are chronic carriers of this virus and approximately 500,000 people die from it each year (Bukh, 2016). In Africa, the prevalence of this disease was estimated at 2.90% between 2000 and 2015 (Petruzziello *et al.,* 2016), and sub-Saharan African countries had an average prevalence of 2.98% between 2002 and 2014 (Rao *et al.,* 2015). In Burkina Faso, HCV is responsible for 900 deaths each year (Zeba *et al.,* 2014), which constitutes a major public health problem. In 2013, the prevalence of anti-HCV antibodies (Ab) in Ouagadougou was 4.4% (Zeba *et al.,* 2014). Transmission of the virus occurs mainly through

blood and transfusions of contaminated blood in many resource-limited countries constitute one of the major transmission source (Tagny *et al.,* 2012).

Therefore, the safety of blood products is a global health priority. The diagnostic approach is different from that of HCV screening, especially in the context of blood transfusion. Indeed, microbiological security consists of eliminating all blood bags positive for HCV due to the presence of anti-HCV antibodies, HCV antigen (Ag) and/or HCV RNA. The nucleic acid amplification test (NAAT), more sensitive (Laperche *et al.,* 2015), in the prevention of HCV transmission by blood transfusion, remains difficult to access in Burkina Faso: thus the detection of HCV Ag, although less sensitive than NAAT (Laperche *et al.,* 2015; Sanz *et al.,* 2002), has been proposed as an alternative to improve the safety of blood transfusions, using ELISA (Enzyme-linked immunosorbent assay) tests for HCV capsid antigen (Ag alone) or by 4th generation tests(Ag/Ac) of HCV.

Screening for hepatitis C at the Regional Blood Transfusion Center of Ouagadougou (CRTSO) is usually carried out with the Monolisa ^{TM} HCV Ag-Ab ULTRA V2 assay (Bio-Rad).

The aim of this study was to compare the performance of the Murex HCV Ag/Ab Combination kit (DiaSorin) with the one of the Monolisa ^{TM} HCV Ag-Ab ULTRA V2 assay (Bio-Rad) for HCV screening in the microbiological qualification of blood donations in Ouagadougou, Burkina Faso.

Materials and Methods

Study setting and framework

The Regional Blood Transfusion Center of Ouagadougou (CRTS/O), the operational unit of the national blood transfusion center (CNTS) was used to collect samples (sera) already analyzed by the MonolisaTM HCV Ag-Ab ULTRA V2 assay (Bio-Rad). The analyses by the Murex HCV Ag/Ab Combination assay (DiaSorin) were carried out in the viral immunology unit of the CHU-YO bacteriology-virology department.

Type and period of study

This was a cross-sectional descriptive study that took place from December 2022 to April 2024 in Ouagadougou.

Study population: sample sources

It consisted 453 of voluntary blood donors received at the fixed CRTSO site and blood donors received by mobile collections teams during the study period.

Non-inclusion criteria

Insufficient serum quantity; Lactescent and hematic serum; Samples whose results for anti-HCV antibody were not specified by the MonolisaTM HCV Ag-Ab ULTRA V2 assay (Bio-Rad)**.**

Collection of samples and socio-demographic data

After non-repetitive blood sampling, the sera were analyzed by the Monolisa ^{TM} HCV Ag-Ab ULTRA V2 assay (Bio-Rad) used routinely at the CRTSO to search for HCV markers. Following this first analysis, 0.5 to 1mL sera were aliquoted into cryovials and stored at -20°C at CHU-YO until analysis by the Murex HCV Ag/Ab Combination assay (DiaSorin). Sociodemographic data of each donor were collected on anonymous survey forms, the numbers of which were correlated with donor identifiers at the CRTSO.

Serological analyses in the laboratory

Sera were analyzed by the Monolisa ^{TM} HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and the Murex HCV Ag/Ab Combination assay (DiaSorin) for the detection of HCV markers, according to the manufacturers' instructions.

The Monolisa $^{TM\ HCV\ Ag\text{-}Ab\ ULTRA\ V2}$ assay (Bio-Rad) that is used at CRTS/O in the context of transfusion safety, is a qualitative immuno -enzymatic test for the diagnosis of HCV, based on the detection of antibodies and capsid antigen in human serum or plasma.

Monolisa ^{TM} HCV Ag-Ab ULTRA V2 assay operating mode:

For each analysis, the protocol proposed by the manufacturer was strictly followed; control of negative and positive sera to validate the quality of the test were used. The sample distribution and identification plan was also carefully established as well as the preparation of the diluted washing solution R2 and the working solution of the positive control antigen (R5a+R5b). The process recommended by the kit was strictly followed:

Remove the support frame and the bars (R1) from the protective packaging.

Place directly, without pre-washing the plate, successively:

- ✓ 100 µL of conjugate 1 (R6) in each well
- ✓ 50 µL of the first sample in F1,
- ✓ 50 µL of the second sample in G1, etc.
- ✓ 50 µL of negative control (R3) in A1,
- ✓ 50 µL of positive control (R4) in B1, C1, D1,
- ✓ 50 µL of the working solution of the positive control antigen (R5a + R5b) in E 1,

Homogenize by 3 aspirations at least or with a microplate shaker for 5 seconds and cover with self-adhesive film.

- Incubate the microplate in a dry microplate incubator for 90 ± 5 minutes at 37°C ± 1°C.
- After 90 minutes, we washed the microplate using an automatic washer (a minimum of 5 washes).
- Dry the plate by turning it over on a sheet of absorbent paper.
- Rapidly distribute 100 µL of conjugate solution 2 (R7) into all wells. The conjugate must be shaken before use. Cover, if possible, with new film and incubate for: 30 ± 5 minutes at 37°C ± 1°C.
- Remove the adhesive film after 30 minutes and wash 5 times as before.
- Prepare the revealing solution
- Distribute 80 µL of the development solution rapidly into all the wells and allow the reaction to develop in the dark for 30±5 minutes at room temperature (18 to 30°C). During this incubation, do not use adhesive film.
- Add 100 µL of the stopping solution (R10) using the same sequence and distribution rate as for the revealing solution.
- Wipe the underside of the plates thoroughly. Wait at least 4 minutes after dispensing the stop solution, and within 30 minutes after stopping the reaction, read the optical density at 450/620-700 nM using a plate reader

Monolisa ™ HCV Ag-Ab ULTRA V2 assay results interpretation:

Samples with an optical density below the cutoff value (determined by taking one-quarter of the mean of the optical density of the positive control) were considered negative by the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad).

Samples with an optical density higher than or equal to the cutoff value were considered initially positive. However, results just below the cutoff value (S/CO-10% <OD<S/CO) which should be interpreted with caution were considered positive.

For the Murex HCV Ag/Ab Combination assay (DiaSorin), It is a two-step enzyme immunoassay for the detection of HCV. Unlike the Monolisa ™ HCV Ag-Ab ULTRA V2 (Bio-Rad), the Murex HCV Ag/Ab Combination (DiaSorin) uses a sandwich system for the antigen and antibody.

Each sample was tested by a monoclonal antibody sandwich used for the HCV capsid antigen plus recombinant NS3 protein and the capsid sandwich used for antibody detection.

Murex HCV Ag/Ab Combination assay (DiaSorin) operating mode

For each analysis, the ELISA kit and the sera to be analyzed were brought back to room temperature, the washing solution was prepared and the conjugate reconstituted.

For each plate, the process was as follows: - Distribute 50 µL of the sample diluent into all wells

- Distribute 50 µL of the samples to be analyzed into the wells starting from well E1 - Add 50 µL of the negative control (NC) into wells A1 and B1)
- Add 50 µL of the positive control for the antibody into well C1
- Add 50 µL of the positive control for the antigen into well D1.
- Cover and incubate and incubate for 60 min at 37°C
- After 60 min, wash the plate using an automatic plate washer (5 washes) - Then invert the plate onto absorbent paper, tapping the back to absorb the residual wash solution.
- Immediately add 120 µL of conjugate to each well, cover and incubate for 60 min at 15-28°C. Immediately add 120 µL of conjugate to each well, cover and incubate for 60 min at 15-28°C.
- Preparation of substrate solution
- After 60 min, wash the plate using the plate washer and invert it on absorbent paper, tapping the back to absorb residual wash solution.
- Immediately add 80 µL of substrate solution (TMB) to each well, cover and incubate for 30 min at 37°C ± 1°C.

Keep away from light.
- Add 50 µL of stop solution.
- Reading of the absorbances 15 min after the addition of the stop solution at 450 nM (620-690 nM) after having evacuated the Elisa device.

Murex HCV Ag/Ab Combination assay (DiaSorin) result interpretation

The Cutoff Value (CV) is calculated by adding 0.2 to the mean absorbance of the negative control assayed in duplicate. Samples giving an absorbance less than the cutoff value were considered non-reactive and those having an absorbance greater than or equal to the cutoff value were considered initially reactive.

Operationally, the overall detection rate was defined as the set of positive cases detected by the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and/or by the Murex HCV Ag/Ab Combination assay (DiaSorin). This definition is based on microbiological safety in blood transfusion: it excludes all bags likely to cause infection by the presence of one or more blood-transmitted agents.

The analytical performance of the Murex HCV Ag/Ab Combination assay (DiaSorin) was evaluated using the routinely used Monolisa TM HCV Ag-Ab ULTRA V2 assay (Bio-Rad) as a reference for CRTS/O.

Data were analyzed using Epi-Info software version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). The significance threshold for comparing two proportions was set at p value <0.05.

Results and Discussion

This study involved 453 blood samples donor including 376 men (83%) and 77 women (17%). Donors were 18 to 60 years aged with an average age of 31.59 ± 8.25 years. The most represented age groups were those from 20 to 30 years with 197 (43.49%) donors and those from 30 to 40 years, with 159 (35.10%) donors in the study population. The profession was not specified in 79.25% (359/453) and mobile collections represented the majority of the samples with 64.68% (293/453). First-time blood donors represented 62.25% (282/453) and 48.12% (218/453) of donors lived in the Central region.

The detection rate of markers by the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and the Murex HCV Ag/Ab Combination assay (DiaSorin) are shown in Table 1. There was no statistically significant difference in HCV marker detection between the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and the Murex HCV Ag/Ab Combination assay (DiaSorin) (p=0.08).

The detection rate of HCV markers was 4.52% and 5.85% in men respectively for the Monolisa ™ HCV Ag-Ab ULTA V2 assay (Bio-Rad) and the Murex HCV Ag/Ab Combination assay (DiaSorin). There was no statistically significant difference between these two assays (p=0.06). They were 2.60% and 3.90% in women respectively for the two kits and there was also no statistically significant difference (p=0.92). We observed that the detection rate was higher beyond 20 years with prevalence's of 3.14 to 7.55% with both assays.

The overall detection rate was 9.05% in the total population based on HCV antigen and anti-HCV antibody detection versus 4.19% and 5.52% respectively for Monolisa TM HCV Ag-Ab ULTRA V2 (Bio-Rad) and for the Murex HCV Ag/Ab Combination assay (DiaSorin) taken separately.

The evaluation of the performance parameters of the Murex HCV Ag/Ab Combination assay (DiaSorin), taking as reference the Monolisa ™ HCV Ag-Ab ULTA V2 assay (Bio-Rad) used routinely at the CRTSO, gave a low sensitivity of 15.79% and a specificity of 94.93%.

The cross-tabulation between the MonolisaTM HCV Ag-Ab ULTA V2 assay (Bio-Rad) and the Murex Ag/Ab combination assay (DiaSorin) and the diagnostic performance of the Murex Ag/Ab combination assay (DiaSorin) are shown in Tables 2 and 3.

Male blood donors were the most numerous (83%) compared to 17% for female donors, with a *sex ratio* of 4.88. Several other authors have made the same observations including four in Burkina Faso and others around the world, Ghana, Cameroon and Kyrgyzstan (Nagalo *et al.,* 2011; Nagalo *et al.,* 2012; Nébié *et al.,* 2007; Ouédraogo *et al.,* 2012; Osei *et al.,* 2017; Karabaev *et al.,* 2017). This could be explained by the fact that contraindications to blood donation (low weight, low hemoglobin level, menstruation, pregnancy and breastfeeding) concern more women than men. The age of blood donors was between 18 and 54 years with a mean age of 31.59 ± 8.25 years. This mean age was higher than those reported by other authors: Ouédraogo *et al.,* (2012); Karabaev *et al.,* (2017) and Tagny *et al.,* (2014) in the literature (Ouédraogo *et al.,* 2012;

Karabaev *et al.,* 2017; Tagny *et al.,* 2014) who found average ages of 28.78±8.9 years, 27 years and 29.20 ±8.0 years respectively.

The HCV detection rates in this study were 4.19% for the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and 5.52% with the Murex HCV Ag/Ab Combination assay (DiaSorin). These results confirm those of Laperche in 2015 who had already compared the two kits using HCV RNA-positive samples with a rate of 38.3% for Monolisa ™ HCV Ag-Ab ULTRA V2 (Bio-Rad) versus 47.5% for Murex HCV Ag/Ab Combination (DiaSorin). This could be explained by the genetic heterogeneity of HCV, the low sensitivity of the Monolisa ™ HCV Ag-Ab ULTRA V2 test (Bio-Rad) for the detection of HCV capsid proteins (Laperche *et al.,* 2005) and the good sensitivity of the Murex HCV Ag/Ab Combination assay (DiaSorin) for genotype 1 and 3 (Laperche *et al.,* 2015). We observed that the two kits did not have the same constitution and the same composition in the kit. This makes us think that the choice of antigens and the presentation of the method of analysis of serum samples could influence the detection rate.

Indeed, the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) uses recombinant NS4, NS3 proteins of genotype 1 and 3a and a mutated capsid peptide and the Murex HCV Ag/Ab combination assay (DiaSorin), recombinant antigens, NS3 peptides and a viral capsid antigen.

The detection rate was higher in men than in women: 4.52% versus 2.60% for the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and 5.85% versus 3.90% for the Murex HCV Ag/Ab Combination assay (DiaSorin). The high detection rate in men could be explained by the fact that the *sex ratio* was in favor of men and also by the fact that male sex would be a factor favoring the evolution of HCV (Leone and Rizzetto, 2005), as well as alcohol consumption (Leone and Rizzetto, 2005).

In Burkina Faso, 52.4% of men consume alcohol compared to 50.2% for women (Boua *et al.,* 2018). On the other hand, the low detection rate in women could be explained by the clearance of HCV, because the female sex, white subjects and the absence of co-infection, especially with HIV are factors associated with spontaneous elimination of the Hepatitis C virus (Broutin *et al.,* 2006). Tagny *et al.,* (2014) reported in Cameroon after confirmation of positive cases, a prevalence of 2% for men and 1.20% for women. Gao *et al.,* (2011) reported a detection rate of 9.87% in men and 9.78% in women.

Concerning the age groups, the detection rate was higher beyond 20 years. Indeed, age is a factor favoring the evolution of HCV (Leone and Rizzetto, 2005). Infection generally occurs after 40 years. Gao *et al.,* (2011) had found a prevalence of 8.99% in subjects aged 31 to 60 years. Karabaev *et al.,* (2017) also obtained high rates in subjects aged at least 30 years. But in general, for the detection rate at the age group level, the Murex HCV Ag/Ab Combination kit (DiaSorin) had a higher rate than that of the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad).

The overall detection rate was 9.05% compared to 4.18% for the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) and 5.52% for the Murex HCV Ag/Ab Combination assay (DiaSorin). This could be explained by the differences between the two kits in terms of their components, their analysis methods and the genetic heterogeneity of HCV. Thus, with this overall detection rate, we could to make blood products safer and reduce the rate of HCV transmission compared to each kit taken individually.

The sensitivity was estimated in the present study at 15.8% with a 95% confidence interval (95% CI) of [4.9-38.6]. It was lower than that of Yang *et al.,* in 2011 which was 100% and that of Laperche *et al.,* (2015) in France (47.5%). This sensitivity was outside the range given by Laperche *et al.,* (2015) which was 29 to 70% for the sensitivity of combined tests compared to NAAT Laperche *et al.,* (2015). We explain this low sensitivity by the non-use of a gold standard such as a nucleic acid amplification test to assess the real performance of the Murex HCV Ag/Ab combination assay (DiaSorin).The specificity of the Murex HCV Ag/Ab combination assay (DiaSorin) obtained in the present study was 94.93% with a 95% CI of [98.9-100]. It was lower than that of Yang *et al.,* (2011) on the diagnostic performance of the combined tests whose specificity obtained was 95.40%. The lack of a gold standard such as a nucleic acid amplification test previously mentioned for sensitivity could also concern the specificity.

Table.1 Detection rates of the two kits

	Monolisa ™ HCV Ag-Ab		Murex HCV Ag/Ab		*p-value*
	Number	%	Number	%	
Negative	434	95.80	428	94.50	
Positive	19	4.19	25	5.52	*0.08*
Total	453 (100)		453 (100)		

Table.2 Cross-tabulation of Murex HCV Ag/Ab Combination (DiaSorin) and Monolisa ™ HCV Ag-Ab ULTRA V2 (Bio-Rad)

Tests	**Monolisa ™ HCV Ag -Ab ULTRA V2 (Bio-Rad)**			
Murex Ag /Ab combination (DiaSorin)		Positive	Negative	Total
	Positive	3	22	25
	Negative	16	412	428
	Total	19	434	453

Table.3 Diagnostic performance of Murex Ag/Ab Combination (DiaSorin)

Performance parameter	**Results**	**95% CI**
Sensitivity (Se)	15.8	[4.9-38.6]
Specificity (Sp)	94.93	[94.4-96.7]
Positive Predictive Value (PPV)	12	[0-24.7]
Negative Predictive Value (NPV)	96.26	[94.5-98.1]

The need for blood products in Burkina Faso is enormous because of children and anemic women. However, HCV transmission is mainly parenteral and blood transfusion is one of the most frequent transmission routes. The safety of blood components has been declared as a goal to be achieved worldwide by World Health Organization (WHO), including microbiological safety. The sensitivity of Murex HCV Ag/Ab combination was very low unlike its specificity. However, the combination of these two assays as a screening technique in blood transfusion gave a higher detection rate and will make it possible to obtain safer blood products and reduce HCV transmission in Burkina Faso and beyond in low-income countries. The discordant results obtained which represented 92.68% could not be confirmed in this study. The re-evaluation of screening assay would be crucial for better microbiological safety in blood transfusion in Burkina Faso.

Current state of knowledge on the subject

- Fourth generation ELISA HCV screening tests (Ag/Ac) improve the microbiological safety of blood products by early detection of HCV compared to previous generation tests.
- For better transfusion safety, the serological or virological window should be as short as possible to reduce the transfusion risk of HCV.

Blood product safety is a global health priority. The study objective was to contribute to improving microbiological safety of blood products in Burkina Faso. Sera from volunteer donors' blood collected at the Regional Blood Transfusion Center of Ouagadougou were tested by the Monolisa ™ HCV Ag-Ab ULTRA V2 (Bio-Rad) and Murex HCV Ag/Ab Combination (DiaSorin) assay. The analytical performances of the two kits were compared. The sensitivity and specificity of Murex were evaluated using the Monolisa ™ HCV Ag-Ab ULTRA V2 assay (Bio-Rad) as a reference. Differences were considered statistically significant for any value of $p < 0.05$. A total of 453 non-repetitive blood donations were tested by both kits. The detection rate of HCV markers by the Monolisa ™ HCV Ag-Ab assay was 4.19% versus 5.52% for Murex HCV Ag/Ab. The difference in detection of HCV markers between the two kits was not statistically significant (p=0.08). The overall detection rate was 9.05%. The sensitivity was 15.8% and the specificity was 94.93%. The detection rate of HCV

markers by the Murex HCV Ag/Ab Combination assay (DiaSorin) was higher than that of Monolisa ™ HCV Ag-Ab ULTRA V2 (Bio-Rad), but this difference was not statistically significant. However, the combination of these two kits in blood transfusion gave a higher detection rate (9.05%) and allows to obtain safer blood products.

Contribution of our study to knowledge

- This study will show that a single fourth-generation screening test is insufficient for the microbiological safety of blood products in blood transfusion in developing countries.
- This postulate will allow the introduction of two fourth generation tests in blood transfusion or the transition to the use of Nucleic Acid Amplification Techniques in developing countries.

Authors' Contributions

Anicet TAMALGO: a collection THE samples, performed the biological analyses and wrote the body of the article. Salam SAWADOGO: authorized the collection of samples. Absatou BA/KY: corrected the article. Harouna BELEM, Agnès BAMBARA: participated in the biological analyses of the samples. Maxime KIENOU: data analysis. Lassana SANGARE: Principal investigator of the work and corrected the article.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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