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Improved specificity of testing for anti-HCV antibodies in CNTS Abidjan by using the DO / VS ratio

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***Abstract*— Hepatitis C is an infectious disease caused by the virus identified no-A, no-B virus called Hepatitis C virus (HCV), which is transmitted by the parenteral route. In Côte d'Ivoire, the prevalence of this disease is higher than 3%, or about 600 000 infected people. HCV represents a public health problem. The most common way of transmission is blood transfusion so that we should improve screening techniques that virus. The National Blood Transfusion Center (NBTC) in Abidjan, in order to limit the risk of transfusion transmission, conducts tests for viral diseases such as those caused by hepatitis C. The ELISA Dia.pro HCV third generation is used for detection. Moreover, this test is highly sensitive because of the derived window period at 6 weeks. There by observe a high false positive rate of reaction. The performance of this test for the ratio R1 = 3 and R2 = 5 to the reference test like RIBA and the performance of the algorithm 1 used were evaluated. The results obtained for the ratio R1=3 gives a sensitivity Se = 100%; specificity Sp = 58,33% and a positive predictive value Vpp = 99,23% with a positive predictive value reference of 95%. The evaluation of the algorithm provides a positive repetitive rate estimated of 84,93%. This study shows that the specificity of the ELISA Dia.pro is improved when the ratio is 5 with a positive predictive value according to the reference value. The best algorithm is also best as the positive rate is repeated close to the reference value which is 95%.**

***Index Terms*—Hepatitis C Virus, ELISA, ratio DO / VS**

I. INTRODUCTION

HCV infection is characterized by a high risk of chronicity with persistent viral replication throughout the course of the disease [1; 2]. Current epidemiological data to assess approximately 130-170 millions the number of chronic carriers of hepatitis C in the world, with an average prevalence of about 2,2% [3]. This prevalence varies from country: very low in Northern Europe, higher in Southeast Asia and Africa, it is reaching more than 20% in Egypt [4; 5]The World Health Organization estimates that nearly 350.000 the number of annual deaths consecutive to infection by the hepatitis C virus [6]. Moreover, there is no vaccine against hepatitis C. Thus, the most effective way to fight against hepatitis C lies in controlling the risk of nosocomial transmission of HCV namely blood transfusions, transplants bodies and unsafe injections such as the use of intravenous drugs [7; 8; 9; 10; 11]. Screening for people at risk, including those who received blood products before 1992, is also an important element in the fight against this infection [12; 13]. 3% of the worldwide population is infected with HCV, 200 million people, according to WHO, with over 350.000 deaths related to hepatitis C. Côte d'Ivoire's prevalence is estimated at more than 3,6% [14] with a seroconversion rate of donors estimated at 1.64%. Also, among the 500 000 to 600 000 subjects infected in France, 80% are viremic. Only a quarter of infected people seem to know their status to HCV [15; 16; 17; 18]. Testing as a means to fight against Hepatitis C virus is therefore important to differentiate asymptomatic apparently healthy from no-HCV-infected people. Furthermore, the magnitude of the infected population and the risk of serious changes in 10 to 30 years make HCV an important public health issue [19; 20; 21; 22]. Improve the specificity of screening tests for hepatitis C by using the optical density ratio / threshold value (OD / VS) is necessary. To do that, the performance of the test for HCV "Dia.pro" will be evaluated. Ratios DO / VS for which the results of this testing are reliable will also be determined.

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II. MATERIALS AND METHODS

This study was conducted over a period of 3 (three) months in the National Blood Transfusion Center (NBTC) of Abidjan. It aims to assess the specificity and sensitivity of the Elisa test Dia.pro for screening hepatitis C in the center. Also highlight the importance of the use of ratios DO / VS to improve specificity for reducing false positive reactions. The size of the sample on this study was set to 80 samples, given the availability of blood bags. The sera were obtained by centrifugation of blood samples. These samples are called positive for HCV ELISA Dia.pro a ratio (optical density / threshold) R1 = 3. As regards the analysis of blood, the Dia.pro HCV Ab ELISA and RIBA 4.0 HCV Deciscan 4.0 more tests were used. These tests are available in kit form containing control solutions, diluents, calibrator, conjugate, substrate, stop solution and washing. A centrifuge BioRad, BioRad IPS incubator, the PW40 washers and a Biorad spectrophotometer connected to both a computer and a printer were used as technical equipment. HCV Ab test Dia.pro third generation version 4.0 was achieved for the detection of anti-HCV antibodies in serum. To do that, a microplate whose wells are coated with the core peptides and recombinant peptides NS3, NS4 and NS5 was used. The conjugate consisting of IgG and IgM goat anti-human immunoglobulin linked to peroxidase. The results of this test were performed by the DO / VS ratio namely the ratio of the optical density on

the threshold value. The RIBA HCV Deciscan test, unit testing membrane allowed the individualization of anti-HCV antibodies in serum. This test is used as a solid support strip adhered to a plastic membrane, which are fixed the various test samples. The evaluation of performance Elisa was performed for each ratio and compared with the EPI Info 6.04 software.

III. RESULTS AND DISCUSSION

The results of screening tests with Dia.pro and RIBA are shown in Table I. The samples analyzed with the Dia.pro test for R1 ratio = 3 are all positive HCV. By cons, with the ratio R2 = 5 three months after giving four of the 80 negative samples tested is a 5% reduction in the rate of positive. Analysis of the samples with the RIBA meanwhile give an effective 68-positive ratio for R1 = 3 with 12 negative samples. Regarding the ratio R2 = 5 for this test, 62 positive and 18 negative is obtained a reduction of 10% of the positive rate. The evaluation of the test gives Dia.pro RIBA positive rate for the ratio R1 estimated at 85% and the ratio of R2 to 77,5%. The following evaluation test Dia.pro ratios R1 and R2 with the reference test RIBA provides the results in Table II. For ratio R1 = 3; 68 true positives were obtained with 5 false positives and true negatives 7. For the ratio R2 = 5; 60 true positives, 3 false positives, 2 false negatives and 15 true negatives are obtained. These results are used to calculate performance namely: sensitivity, specificity and positive predictive value of the test on Dia.pro ratios
(see Table III). For the ratio R = 3 sensitivity reaches its maximum value is 100%. However, the specificity is very low (58,33%). Regarding the ratio R2 = 5, test sensitivity decreases by 5%, but specificity increases to 25%. Specificity is best for R = 5. The positive predictive value increased from 93,15% to R1 = 3 and 95,23% for R2 = 5.

TABLE I: Results of Screening of both tests

		DIA.PRO	RIBA
R = 3	Positive	80	68
	Negative	0	12
R = 5	Positive	76	62
	Negative	4	18

Table II: Results of test performance Elisa Dia.pro according to the ratio

			RIBA TEST		
			Positive	Negative	Total
DIA.PRO TEST	R = 3	Positive	68	5	73
		Negative	0	7	7
	R = 5	Positive	60	3	63
		Negative	2	15	17

Table III: Summary of test performance Elisa Dia.pro.

	Se	Sp	Vpp
R1 = 3	100%	58,33%	93,15%
R1 = 5	96,77%	83,33%	95,23%

The calculation of the performance of the algorithm 1 is summarized in Table IV ratios R1 and R2 are used. The samples analyzed with respect to ratios give the following results: 7 samples negative for the ratio R1 = 3 and R2 = 5; 62 positive samples for R1 and R2; 11 samples positive for the ratio R1 and negative for R2. Therefore these values are used to calculate the rate of repetitive positive and the rate of repetitive negative using the following formulas:

$$PR = \frac{\text{Positives (R1 et R2) x 100}}{\text{positives + negatives}}$$

$$NR = \frac{\text{Negatives (R1 et R2) x 100}}{\text{Negatives (R1 et R2)}}$$

Thus, the repetitive positive rate (PR) is PR = 62 x 100/73 = 84.93%. With a repetitive rate of negative (NR) which is NR = 7 x 100/7 = 100%. The rate of positive repetitive 84,93% is 10% lower than value recommended by the Center for Prevention and Control of Diseases [5].

Table IV: Calculation of performance of Algorithm

		Dia.pro 2 months DO/VS at 3 months	
		< 5	**> 5**
Dia.pro 1 DO/VS	< 3	7	0
	> 3	11	62

According to the work of Rotily and collaborators, the sensitivity of a screening test is important because it eliminates all doubtful samples [13]. This study indicates that a ratio R1 = 3, the first-line test is better with a sensitivity of 100%. Morretti and collaborators [22] were obtained for this ratio, a sensitivity equal to roughly 99.5%; which confirms this result. Previous studies by DIBY, at the NBTC on 2013 [23], also corroborate this result. However, the specificity of Elisa test for anti-HCV antibodies, for the ratio R1 = 3, is very low (Sp = 58%). This test can be recommended for first-line screening for its satisfactory sensitivity. Three months later, when serological testing is done with the ratio R1 = 3, no seroconversion was observed. The result remains unchanged. When the control is done with R2 = 5, the results show a slight decrease in sensitivity and a very large increase in the specificity of the test Dia.pro. Sp (R2 = 5) equal 83,33%

higher than the value Sp (R1 = 3). This ratio indicates a better test specificity Elisa Dia.pro; positive predictive value increases from 93,15% to R1 = 3 and 95,23 for R2 = 5. 95% is recommended by the CDC qualitative value of the positive predictive value [26; 27]. Also, the positive predictive value for the ratio R2 = 5, gives satisfactory results. The Elisa test Dia.pro for R2 = 5, becomes more specific. We then deduce that the specificity of this test increases with the ratio. These results are confirmed by Dufour's studies and collaborators [25]. They have evaluated the specificity of the diagnostic test Elisa orthoclinical to 97,3% for R2 = 5, and a CLIA assay (VITROS ECI; Ortho Clinical Diagnostics), to 99,2%. For Vpp equal 95,3% and a specificity equal 83,33%, the Dia.pro Elisa test is recommended for second-line tests with a ratio R2 = 5. This allows us to observe a decrease of the rate of false positives. Evaluation of the Algorithm 1 in this study, provides a repetitive rate equal 84,93% positive. This rate is close to the rate of repeat positive recommended by the CDC, which is 95%. This value allows us to say that the logarithm 1 can be suggested for testing second-line.

IV. Conclusion

Hepatitis C is a viral disease transmitted parenterally. Transfusion is the transmission channel observed most of the virus; therefore it is important that effective screening is carried out on blood products to prevent contamination of transfused people. Sensitive serological tests such as ELISA test must be confirmed by specific tests called confirmatory tests that reveal the presence of this virus in the blood plasma: they are, for example, PCR and RIBA. The performance evaluation of the test on Elisa Dia.pro considered different ratios R1 = 3 and R2 = 5, after checking, we can remember that the ratio increases with specificity. Indeed, the specificity of the test for R = 3, pass by 83,33% to 58,33%, for R = 2, the positive predictive value increases by 95,23% to 93,15%. The positive predictive value is better than 95%, the ratio R2 = 5 is the ratio that highlight the specificity of the test Elisa Dia.pro. The adopted Algorithm 1 for the evaluation of performance on the Elisa test Dia.pro ratio, has a repetitive positive rate equal 84,93%. This value is close to 95%, the recommended value.

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