

# Original Research Article

## **Influence of Rheumatoid Factors and Bilirubin on the Sensitivity of Immunochromatographic Rapid Diagnostic Tests for HIV/AIDS and Hepatitis B in Cameroon.**

### **ABSTRACT:**

**Aims:** This study aimed at identifying some potential interference factors in the immunochromatographic Rapid Diagnostic Tests commonly used in Cameroon for the diagnosis of Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) and Hepatitis B (HBV) which are major public health problems in the country.

**Design and Methods:** The sample population included patients referred to BETHANIE Laboratory for the accurate diagnosis of HIV/AIDS and HBV. RDTs were performed using FIRST RESPONSE HIV Card 1-2.0 and HEXAGON HBsAg. Tests results were confirmed using a high sensitivity 3<sup>rd</sup> generation ELISA for HIV and HBV, both from FORTRESS. Assays of Rheumatoid Factors and bilirubin were conducted on HIV and HBV samples respectively. Statistical analysis was done using the R software version 3.0.2; the Kappa test and ANOVA were applied at a threshold of 0.05. Graphs were plotted using Microsoft Excel 2010.

**Results:** A total of 25 patients were included for the HIV study group and 30 for the HBV group. Test sensitivities of 14.28% and 92.85% for FIRST RESPONSE HIV Card 1-2.0 and HEXAGON HBsAg were found respectively.

**Conclusion:** Rheumatoid Factor (up to 238.8 UI/l) was not found to interfere with the performance of HIV FIRST RESPONSE RDT ( $p$ -value > 0.05) and there was no interference of bilirubin (up to 22.8 mg/dl) with the performance of HEXAGON HBsAg RDT ( $p$ -value > 0.05).

**Key words:** Interference, Rapid Diagnostic Test (RDT), HIV/AIDS, Hepatitis B (HBV), Rheumatoid Factor (RF), Bilirubin.

### **1. INTRODUCTION**

Advances in Biotechnology have led to the development of rapid diagnostic tests (RDTs) for the early and rapid diagnosis of certain diseases which constitute a serious public health burden in resource-limited settings. An accurate diagnosis is very important because it would facilitate proper treatment and possibly lead to a total recovery of the patient[1]. Adequate diagnosis leads to the proper use of drugs and consequently avoids drug resistance. It may be useful in providing guidance

34 on the choice of drugs as in the case of antibiotics resistance testing. It is equally useful in guiding  
35 healthcare providers on when to stop and possibly change a particular treatment regimen. Thus,  
36 diagnosis occupies a primordial place in the health system.

37 Suitable **diagnosisrequires** appropriate techniques which can lead to the early identification of  
38 an illness. In biological diagnosis, there are two kinds of tests: the direct test which detects some  
39 particles of the germ (antigens or nucleic acid material) and the indirect test which detects the  
40 presence or the previous existence of the germ in the body (antibodies).

41 Many biological diagnostic techniques exist worldwide, **and** which require sophisticated  
42 equipment and skilled staff. These resources are **yetnot** always available in developing countries  
43 (DCs). However, with the development of Biotechnology, RDTs have evolved and taken a leading  
44 position in applied Biology. In contrast to conventional diagnostic methods, RDTs can enable the  
45 diagnosis of an infectious disease within a short time [1]. They have in fact existed for the past three  
46 decades, especially for the detection of HIV/AIDS and malaria. Their use has gradually spread to other  
47 diseases [1].

48 More recently, single step rapid tests have been developed and marketed. More commonly  
49 called “soap tests” or “strip tests,” they are based on the principle of immunochromatography on a  
50 membrane or strip with pre-colored particles [2].

51 For the detection of antigens, the test sample is deposited at one end of a nitrocellulose  
52 membrane. If the desired antigen is present, it binds with specific antibodies labeled with colloidal  
53 gold. Under the effect of lysis-migration-buffer, the antigen-antibody complex migrates by capillarity  
54 and is arrested by a capture antibody attached to the membrane. A positive result is denoted by the  
55 appearance of a colored test line. An internal control is used to validate the test [3].

56 For the detection of antibodies, serum sample or whole blood is placed at one end and then  
57 migrates to an area where the antibodies bind to an antigen conjugated to a developer of selenium  
58 colloid type. This mixture continues to migrate towards the immobilized antigen on the membrane at  
59 the test window. The antibody-antigen **complexspecifically** binds to the antigen and is immobilised,  
60 forming a colored line. In the absence of specific antibodies, the antigen-colloid conjugate migrates  
61 through the test zone area without producing a signal [4].

62 Nowadays, RDTs are widely used in DCs like Cameroon due to their potential comparative  
63 advantage created by the accrued lack of resources, though they also have the highest burden of  
64 most endemic and infectious diseases such as HIV/AIDS and Hepatitis B. The above-mentioned  
65 diseases are serious public health problems in Cameroon. RDTs are commonly used for the early  
66 diagnosis of these diseases throughout the national territory. However, their results often need to be  
67 confirmed by another method. A positive result does not exclude the presence of other pathogens that  
68 are not targeted by the test and a negative result does not exclude the presence of the target  
69 pathogen tested [4].

70 These RDTs are often subject to interference, leading to false results (false positives and/or  
71 false negatives). In this study, we aim to look for possible interferences on the RDTs for two viral  
72 diseases, one caused by an RNA virus (HIV/AIDS) and the caused by a DNA virus (Hepatitis B).

73

## 74 2. MATERIAL AND METHODS

### 75 2.1. Study Area

76 The recruitment of subjects was conducted in the city of Yaounde, Cameroon; and the site of  
77 choice was the BETHANIE Laboratory for its proximity to some referral hospitals in the city and their  
78 affluence in patients. The study period ran from August to November 2013.

### 80 2.2. Study population

81 Patients visiting the BETHANIE Laboratory for the RF test or who had jaundice and agreed to  
82 participate in the study by signing the informed consent or assent forms after clear explanations and  
83 answers to their questions

### 84 2.3. Laboratory Investigations

#### 85 2.3.1. Rapid Diagnostic Tests

86 The RDTs for Hepatitis B (HBsAg HEXAGON) and AIDS (FIRST RESPONSE HIV1-2.0) were  
87 performed using the sera of patients who came to the laboratory with jaundice or requested a RF  
88 examination respectively.

#### 90 2.3.2. Confirmatory Tests

91 ELISA FORTRESS HIV 3rd generation and high sensitivity HBV assays were used for the  
92 confirmation of HIV and HBV respectively.

#### 94 2.3.3. Biochemistry assays

95 The determination of bilirubin (spectrophotometry) and the titrating of RF (turbidimetry) were  
96 performed on the sera of patients with jaundice and those who came for a RF examination  
97 respectively. BIOLABO reagents were used for the determination of bilirubin and FORTRESS  
98 DIAGNOSTIC for the determination of RF

#### 100 2.3.4. Quality Control

101 Quality control was done for each assay. Positive and negative controls for the ELISA tests  
102 and for the "BIOLABO EXATROL-N" (level I) REF: 95010 LOT: 111215C were included. All the  
103 controls were in the range pre-established by the manufacturer.

### 105 2.4. Data collection

106 A questionnaire was used for data collection. Names, age, sex, address, neighborhood and  
107 number of sexual partners during the last six months were recorded.

### 109 2.5. Data Analysis

110 The results were analysed with the R version 3.0.2 software from which the sensitivities, specificities,  
111 efficiencies, positive and negative predictive values were calculated in addition to the determination of

characteristics of the population such as average age and sex ratio. The ANOVA test was done at a threshold of 0.05 as well as the Kappa test. Graphs were plotted using Microsoft Excel 2010.

### 3. RESULTS

#### 3.1- HIV/AIDS Assay

##### 3.1.1. Sample size and socio-demographic data

The characteristics of patients enrolled in the HIV/AIDS arm of the study is presented in Table 1 below. Table 2 presents the RDTs and ELISA results from specimens of the group. Socio-demographic data and RDTs and ELISA results profile of patients enrolled in the HBV arm are presented in Tables 3 and 4 respectively.

Table 1. HIV/AIDS distribution based on gender

| SEX    | Numbers | Percentage | Positives | Negatives |
|--------|---------|------------|-----------|-----------|
| Female | 14      | 56.00      | 02        | 11        |
| Male   | 11      | 44.00      | 05        | 06        |
| Total  | 25      | 100        | 07        | 17        |

Sex ratio (female/male): 1.27.

The patients were of a mean age of 47.36 years, with the majority being of the 47.36±18.27 year interval. The youngest patient was 9 years old and the oldest 80 years. Of the 14 females, 2 (14.28%) were positive while 11 (78.57%) were negative and 1 (7.15%) was borderline. Out of the 11 males, 5 (45.45%) were positives while 6 (54.55%) were negatives.

##### 3.1.2. RDT and ELISA

For each sample, a RDT was conducted. This was followed by a confirmation with a 3<sup>rd</sup> generation ELISA test.

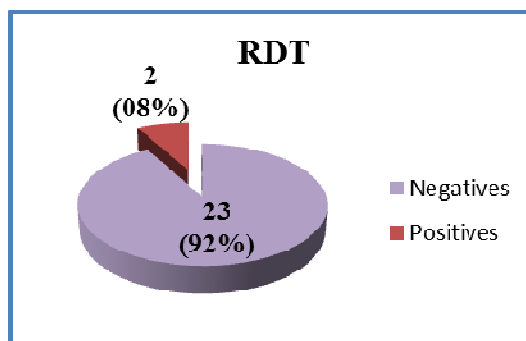


Figure 1a: RDT results

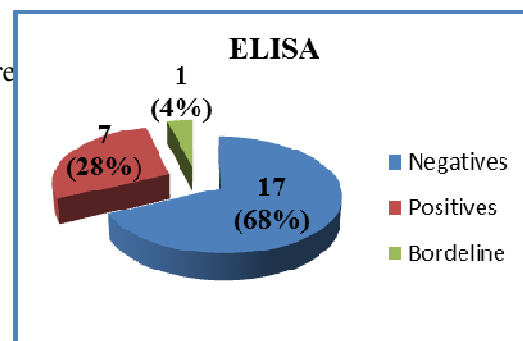


Figure 1b: ELISA results

Using the RDT, 02 (08%) of the 25 blood samples were positive for AIDS and 23 (92%) were negative. By the ELISA, 7 (28%) of the total samples were found positive, 17 (68%) negative and 1(4%) was borderline

##### 3.1.3. Comparison of the FIRST RESPONSE HIV1-2.0 Card RDT with ELISA

RDTs and ELISA were assessed for each specimen. The combined results are presented in Table 2 below.

**Table 2. HIV/AIDS combined results of RDTs and ELISA**

|          | RDT | POSITIVE   | NEGATIVE   |
|----------|-----|------------|------------|
| ELISA    |     |            |            |
| POSITIVE |     | 01<br>(TP) | 06<br>(FN) |
| NEGATIVE |     | 01<br>(FP) | 16<br>(TN) |

Where TP: True Positive, TN: True Negative, FP: False Positive and FN: False Negative

One false positive case was found with a low level of RF (10.6 UI/l). The patient who had a high level of RF (238.8 UI/l) had concordant results with both tests. The sensitivity, specificity, efficiency, PPV and NPV were 14.28 %, 94.11 %, 70.83 %, 50.00 % and 72.72 % respectively.

#### **3.1.4. Correlation between a high RF blood level and false positive result.**

Considering our null hypothesis ( $H_0$ ) that "RF had no effect on the performance of our RDT" and a threshold of 0.05, a two-factor ANNOVA test was used to see if RF had an effect on the RDT of HIV/AIDS. With a  $p$ -value  $> 0.05$ , the null hypothesis ( $H_0$ ) could not be rejected.

### **3.2. Hepatitis B Assay Results**

#### **3.2.1. Sample size and socio-demographic data**

**Table 3. HBV distribution based on gender**

| SEX    | Number | Percentage | Positive | Negative |
|--------|--------|------------|----------|----------|
| Female | 08     | 26.67      | 03       | 05       |
| Male   | 22     | 73.33      | 11       | 11       |
| Total  | 30     | 100        | 14       | 16       |

Sex ratio (female/male): 0.36.

The patients were of a mean age of 39.83 years, with the majority found within the age interval of  $39.83 \pm 18.33$  years. The youngest patient was 13 years old and the oldest was 79. Of the 8 females, 3 (37.5%) were positive while 5 (62.5%) were negative, and 11 (50%) of the 22 males were positive while 11 (50 %) were negative.

#### **3.2.2. RDT and ELISA**

A RDT was conducted on each sample and then confirmed by a high sensitivity ELISA. The results are presented in the Figures 2a and 2b.

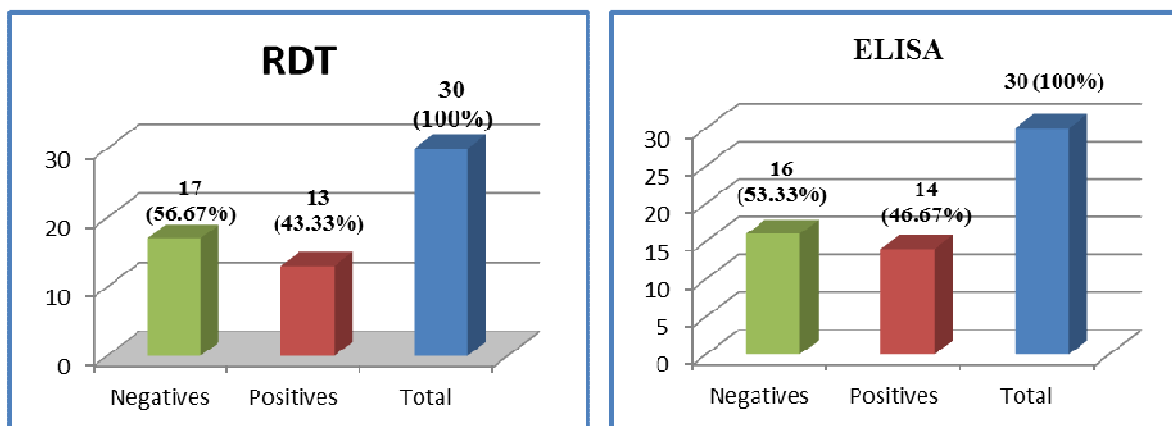


**Figure 2a: RDT results**

**Figure 2b: ELISA results**

**Figure 2: Hepatitis B RDTs and ELISA assays results**

Using the RDT, 13 (43.33%) of the 25 blood samples tested positive for AIDS and 17 (56.67%) were negative. By the ELISA, 14 (46.67%) of the total samples were found positive and 16 (53.33%) were negative.

### 3.2.3. Comparison of the HEXAGON HBsAg RDT with ELISA

RDTs and ELISA were assessed for each specimen; and the combined results are presented in the Table 4.

**Table 4. HBV combined results of RDTs and ELISA**

|          | RDT | POSITIVE   | NEGATIVE   |
|----------|-----|------------|------------|
| ELISA    |     |            |            |
| POSITIVE |     | 13<br>(TP) | 01<br>(FN) |
| NEGATIVE |     | 00<br>(FP) | 16<br>(TN) |

Where TP: True Positive, TN: True Negative, FP: False Positive and FN: False Negative

Just one case was a false negative with a high level of bilirubin (22.8 mg/dl). The sensitivity, specificity, efficiency, PPV and NPV were 92.85%, 100.00%, 96.67%, 100.00% and 94.11% respectively.

### 3.2.4. Correlation between high bilirubin blood level and false negative result.

Setting out from the  $H_0$  hypothesis that "bilirubin has no effect on the RDT," at threshold of 0.05, we used a two-factor ANOVA test to see if bilirubin had an effect on the hepatitis B RDT. With a  $p\text{-value} > 0.05$ , this  $H_0$  hypothesis could not be rejected.

## 4. DISCUSSION:

### 4.1.HIV/AIDS

This study focused on comparing the performance (sensitivity, specificity, efficiency, PPV and NPV) of the HIV FIRST RESPONSE RDT with a 3<sup>rd</sup> generation ELISA which is the gold standard, and to evaluate the possible interference of rheumatoid factor with the RDT results . It must be recalled that this RDT has been approved for use in Cameroon by the Ministry of Public Health.

This part of the study involved 25 patients. This sample size was lower than in other studies comparing an HIV RDT with ELISA, as by OonTeket *et al* in 2012 [5] with 994 patients for the OraQuick ADVANCE Rapid HIV 1/2 Antibody Test; Zachary *et al* in 2012 [6] with 4458 patients for the OraQuick<sup>®</sup> RDT; Kroidiet *et al* in 2012 [7] with 15,000 patients for the Determine RDT. These studies had bigger sample sizes primarily because of their durations (years). Secondly, this was not an HIV prevalence testing study, and so our sample size was calculated based on the prevalence of RF which was our parameter of interest. The sample size was therefore adequate.

We found overall sensitivity, specificity, efficiency, PPV and NPV of 14.3%; 94.1%; 70.8%; 50.0%; 72.7% respectively. The sensitivity and specificity found werelower than for Determine RDT which had 100% and 97.9% respectively [7] and for STAT-PAK RDT with 99.7% and 99.3% respectively [7]. The sensitivity turned out to be very much lower than that specified in the reagent leaflet (100%), and was due to the great number of false negatives.

The only false positive case found had a low value of RF (10.7 UI/l). However, our statistical analysis showed no effect of this parameter on the HIV FIRST RESPONSE RDT. It has been shown that a concentration of RF up to 238.8 UI/l does not create cross reactions which would lead to false positive results (Avila *et al.*, 2002). Our results were in contrast with a study in Sao Paolo, Brazil, which showed that RF values greater than 160 IU/l would lead to cross-reactions on a RDT for malaria, resulting in false positives [8]. However, according to the ANOVA test used to find a possible effect of RF on our RDT, we found a *p-value* > 0.05 for a threshold of 0.05 so our hypothesis could not be rejected which stated that there is no effect of RF on the performance of our RDT.

### 4.2.Hepatitis B

This study focused on comparing the performance (sensitivity, specificity, efficiency, PPV and NPV) of the HEXAGON HBsAg RDT with a high sensitivity ELISA (the gold standard) and to evaluate the possibility of interference ofbilirubin on the RDT results.

For this,the 30 samples usedwere lower in number than those forsimilar comparative studies. Franzecket *et al* in 2013 [9] used 272 samples for Determine HBsAg while Maityet *et al* in 2012 [10] had used 100 samples for Crystal HBsAg and SD BiolineHBsAg comparisons with ELISA. However our sample size was not focused on HBV prevalence but on the prevalence of jaundice in HBsAg positive patients (high bilirubin blood level was our parameter of interest). On this basis, our sample size was higher than the required calculated minimal sample size of 16 samples.

We found overall sensitivity, specificity, efficiency, PPV and NPV of 92.8%, 100%; 96.6%; 100%; 94.1% respectively. The values of the sensitivity, specificity, PPV and NPV were lower than those found using Crystal HBsAg and SD BiolineHBsAg with 100% for both [10]. They were also

higher for Determine HBsAg, with 96%; 100%; 99.5% and 100% respectively [9]. Our sensitivity and specificity were also lower than those specified in the reagent leaflet (100% and 99.7% respectively). With a PPV of 100% (which is the best value for consulting health professionals as it best correlates the diagnosis with the patient's condition), HEXAGON HBsAg RDT is adequate for good diagnosis in our epidemiological setting.

One false negative was found among our specimens, with a high blood level of bilirubin (22.8 mg/dl). It is possible that this high bilirubin level may be responsible for the false negative results (100%) by decreasing the sensitivity of the test. This result correlates with that of Asanghanwaet *al* in 2012 [11] who found that bilirubin levels above 30 mg/dL in the sample decreased the concentration of C-peptide by about 10% in the ELISA technique. However, according to the ANOVA test used to determine the possible effect of bilirubin on our RDT, we found a  $p$ -value  $> 0.05$  for a threshold of 0.05. Therefore we could not reject the hypothesis which stated that bilirubin has no effect on the performance of the HBsAg RDT.

## 5. CONCLUSION

From the demographical data, the results of our study, it was found that Rheumatoid Factor does not interfere with the performance of the HIV FIRST RESPONSE RDT for HIV detection ( $p$ -value  $> 0.05$ ). However, the PPV and low sensitivity of the FIRST RESPONSE HIV Card 1-2.0 test brings to question the conditions under which it was approved for use in the country. Nonetheless, our sample size does not permit us to make a conclusive recommendation on this point. Also, the HEXAGON HBsAg RDT was found to be adequate for the diagnosis of viral Hepatitis B in our epidemiological setting as there was no recorded interference of bilirubin with its performance ( $p$ -value  $> 0.05$ ). However, the many other factors that could contribute to the poor performance of these immunochromatographic RDTs need to be investigated and taken into account while they are being validated as vital tools for the diagnosis of many diseases in Cameroon.

## CONSENT

Informed consent was sought and obtained from each patient visiting the BETHANIE Laboratory, before his/her involvement in the study.

## ETHICAL APPROVAL

Ethical clearance N° 2013/11/379/L/CNERSH/SP was obtained from the National Ethics Committee on Research for Human Health (CNERSH) of Cameroon. Research Authorizations were obtained from the Directorate of the School of Health Sciences, Catholic University of Central Africa and the directorate of BETHANIE Laboratory.

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