



# **Syphilis Seroprevalence among Blood Donors at the Chad National Blood Transfusion Center in N'Djamena**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MD Initiator and designer of the research project, Preliminary writing of the article, Critical review and decisive contribution to intellectual content, Final approval of the version to be published, Assumes accountability for all aspects of the research including the accuracy and integrity of any part of the article. - Substantial contributions to the drafting of research methods. Author BN Control and monitoring of laboratory test, Acquisition, statistical analysis and interpretation of data, Preliminary writing of the article, Critical review and decisive contribution to intellectual content, Final approval of the version to be published, Assumes accountability for all aspects of the research including the accuracy and integrity of any part of the article. Author AMM Preliminary writing of the article, Final approval of the version to be published, Assumes accountability for all aspects of the article. Author SZ substantial contributions to the supervision of the execution of laboratory tests and Assumes accountability for all aspects of the article. Author BBO critical review and decisive contribution to intellectual content, final approval of the version to be published, assumes accountability for all aspects of the research including the accuracy and integrity of any part of the article. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JAMMR/2021/v33i1030914

### Editor(s):

(1) Dr. Emmanouil (Manolis) Magiorkinis, General Hospital for Chest Diseases "Sotiria", Greece.

(2) Dr. Mohamed Essa, Sultan Qaboos University, Oman.

(3) Dr.Chan-Min Liu, Xuzhou Normal University, China.

### Reviewers:

(1) Yasser Abdrehim Ghaleb, Yemen.

(2) Vanesa Scholl, Argentina.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67972>

**Original Research Article**

**Received 27 February 2021**

**Accepted 04 May 2021**

**Published 07 May 2021**

## **ABSTRACT**

This involves evaluating the risk of syphilis transfusion in blood donors having a reaction to the group antigen and confirming positive or negative by the treponemal test.

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From August 2019 to August 2020, an observational study of syphilitic markers was conducted with blood donors (replacement families and volunteers non-remunerated) at the Center National de Transfusion Sanguine de N'Djamena. The donors included were also tested negative for the markers (HBsAg, Ab anti HCV, Ag /Ab HIV) according to standard methods of clinical microbiology. During one year, 24587 donors were identified of which 654 (2.65%) were confirmed positive by the treponemal test. Different positive rates of syphilis were determined in family replacement donors (94%), voluntary non-remunerated donors (6.12%), male (86%) and female (13%) respectively. This study determined a level of specific antigen for syphilis in a study population that reacted positively to the non-treponemal test. In view of this result, we recommend screening with the treponemal test in any blood donor having a reaction with a non-treponemal test in order to minimize the residual risk of transmission of syphilis among blood donors in Chad.

**Keywords:** Seroprevalence; syphilis; blood donors; CNTS; N'Djamena.

## 1. INTRODUCTION

Syphilis is a sexually transmitted infectious venereal disease caused by the spirochete *Treponema pallidum*. It constitutes a major problem in blood transfusion safety in the world and also in our country. Rare in industrialized countries in the 1990s, its resurgence since 2000 has been confirmed in Europe and the USA [1].

According to the World Health Organization (WHO), an estimated 12 million new cases of syphilis are reported each year, over 90% of them in the developing country [2]. In developing countries, particularly in sub-Saharan Africa, the incidence of syphilis is unknown because the available studies are seroprevalence surveys conducted in specific populations (pregnant women, blood donors, hospital patients) or populations at risk (sex workers).

In Morocco in 2016, the rate of syphilis among women was estimated at 0.56% in the age group 15 to 49 years [3].

At the Bamako CNTS, the rate of syphilis is estimated at 0.1% in the blood donor population, which consists of 80% of subjects aged 18 to 25 [4].

In Central Africa, particularly Cameroon and the Central African Republic (CAR), the rate of syphilis among blood donors was 4% and 4.36% respectively [5,6].

In Chad, according to sentinel surveillance data collected from pregnant women seen in antenatal care (ANC) in 15 sites in 2013, the rate of syphilis was estimated at 3.3% [7].

In 2015, a study carried out at the National Blood Transfusion Center (CNTS) in N'Djamena among recipients found a percentage of syphilis which was estimated at 4% [8].

From 2017 to 2019, the annual reports on the transmission of syphilis in the CNTS and in Chad in general among blood donors were: 3.11%, 4.86%, 4.14% and 3.24%, 3.67%, 3.25% respectively.

Routine screening for Syphilis Specific Antigen on all donated blood has dramatically reduced the risk of *Treponema pallidum* transfusion infection. However, there remains a residual risk of transmission of *Treponema pallidum*, this risk could be:

- A technical error;
- A new *Treponema pallidum* variant not recognized by certain reagents;
- A blood donation by a recently infected subject (silent period).

Syphilis in blood donors appears to be a threatening reality in the transfusion environment among other infectious agents. Its threat is real and requires special attention.

Our study was designed to determine the percentage of the specific antigen of syphilis among blood donors tested positive for the group antigen in Chad in N'Djamena CNTS. Also indicate the most appropriate syphilis screening strategy (s) in blood transfusion [9,10].

The results of this work could be a good awareness-raising tool with a view to actively participating in the fight against syphilis in the search for maximum safety in the transfusion

process and the establishment of a hemovigilance system in Chad.

## 2. MATERIALS AND METHODS

### 2.1 Study Period

This was a one-year, descriptive cross-sectional study from August 2019 to August 2020.

### 2.2 Study Population

It was made up of all the family replacement donors (DFR) and voluntary non-remunerated donors (VNRD) who came during the study period.

All donations were systematically screened for the four markers (HIV, HCV, HBsAg and Syphilis), according to the procedure underway at the CNTS. All donors who donated positive for syphilis were included for the study.

#### 2.2.1 Inclusion criteria

All donors with positive syphilitic serology and informed consent to participate in the study were included in this study.

#### 2.2.2 Non-inclusion criteria

Donors not screened positive for syphilitic serology are not included.

#### 2.2.3 Sample size

All donors who presented during the study period were screened by a first non-treponemal test (794 individuals). Then the confirmatory treponemal test excluded 140, so ultimately 654 subjects made up the size of our sample.

#### 2.2.4 Variables studied

The study variables were as follows:

- Sociodemographic variables: sex, age, profession, marital status, type of donor, donor status.
- Biological variables: non-treponemal test and treponemal test.

#### 2.2.5 Screening algorithm

The syphilis screening was carried out as a 1st intention by a non-treponemal test (Alere determines syphilis) and the positive samples

were then confirmed by a 2nd treponemal test (TPHA).

### 2.2.6 Preparation and storage of samples

Samples were collected on pilot tubes soaked in EDTA. These samples are centrifuged at 3000 rev / min for 5 min immediately after collection to separate the blood serum from the red blood cell pellet. The serum thus obtained is used immediately for testing if there are reagents available. Otherwise, the sera are stored in freezing at less than 25°C while awaiting the availability of the tests.

### 2.2.7 The non-treponemal test

Alere determines syphilis was used as a non-treponemal test for screening donors.

### 2.2.8 Alere's biological principles determines syphilis

Alere determines syphilis TP is an immunochromatographic test for the qualitative detection of antibodies directed against the antigens of *Treponema pallidum*.

### 2.2.9 Alere test procedure determines syphilis

The serum is distributed at 50 µL using a precision pipette on the sample deposition zone symbolized by an arrow.

The reading should be taken at least 15 minutes after the analysis.

### 2.2.10 Interpretation

The results of the screening test are expressed qualitatively: negative (-); invalid and positive (+) following the manufacturer's instructions described on the package insert.

### 2.2.11 Confirmatory treponemal test

TPHA was used as a treponemal test in confirming results.

The TPHA is an indirect hemagglutination test for the qualitative and quantitative determination of specific antibodies to *Treponema pallidum* in human serum.

### 2.2.12 Presentation - conservation – validity

Presentation: 5 dropper bottles

- Storage: Between + 2 to 8° C until the expiration date.

- Validity: In the absence of contamination, they are stable for up to 6 weeks after opening at a temperature between 2 to 8° C.

### 2.2.13 Qualitative determination

1. Using a micropipette, dispense 100 µL of diluent into well 1 of the plate and 25 µL into each of wells 2 and 3.
2. 25 µL of serum sample is added to well 1. The contents of well 1 are mixed with a micropipette and 25 µL from well 2 is transferred to well 3 (test well). 25 µL of liquid from well 3 are mixed and discarded.
3. 75 µL of well homogenized control cells are added to well 2 and 75 µL of well homogenized test cells to well 3.
4. The liquid is mixed thoroughly in all wells by shaking the plate gently.
5. Place the plate on a flat, smooth white surface, away from sunlight and vibrations. The result is interpreted after 45 to 60 minutes.

## 2.3 Interpretation of Qualitative Results

### 2.3.1 Negative result

Clear pellet of non-agglutinated red blood cells, without any hole or with a very small hole in the middle.

### 2.3.2 Unspecified result

Red blood cell pellet with a small hole in the middle (thick and tight ring on a light background). In this case, we repeat the test.

### 2.3.3 Positive result

Partial or complete agglutination of the red blood cells is observed (homogeneous film, possibly bordered by a circle of red blood cells). Weak positive samples show a frayed ring bordered by clumped red blood cells. In this case, a titration is carried out on the positive result.

## 2.4 Quantitative Determination

1. As described above, the diluent is dispensed, but continues to transfer 25µL also from well 4 until the desired titration.
2. We do not discard the 25 µL of liquid from well 3, but transfer them to well 4, mix and transfer 25 µL from well 4 to well 5, and so

on. Finally, the 25 µL of the last dilution is discarded.

3. 75 µL of control cells are added in well 2 and 75 µL of test cells in well 3 up to the last well.
4. The manipulation is continued as for the procedure for the qualitative determination.

### 2.4.1 Interpretation of quantitative results

The presence of agglutination in the wells indicates a positive result provided that no agglutination is present in the control well.

The title is defined as a last dilution (maximum dilution) where agglutination is still observed (well 3, 1/80; well 4, 1/160; well 5, 1/320, well 6, 1/640 etc.). Samples reacting with *T. pallidum* antibodies at titers  $\geq 1 / 80$  should be considered positive.

## 2.5 Data Processing

The data for the study had been collected using an anonymous standardized survey form comprising the variables considered.

Data were entered and analyzed using Microsoft Word and Excel 2013.

The chi-square test ( $\chi^2$ ) was used for the comparison of qualitative variables with a significance level set at 0.05.

## 3. RESULTS AND DISCUSSION

Of the 24587 donors participating in this survey, 794/24587 (3.23%) were detected positive by the non-specific test, of which 654/24587 (2.65%) were confirmed positive by the treponemal test (TPHA) and 140 donors were declared negative by the TPHA test.

Female donors were (14%) and 86% were male.

The family replacement donors participating in the survey were (94%), against (6.12%) voluntary non-remunerated donors.

In our study, the proportion of syphilis in 654/24587 blood donors was 2.65%. According to several data in the literature, the seroprevalence of syphilis in blood donors varies considerably from one region of the world to another, and even within the same country, from one region to another, from one province. to the other [11].

This rate is higher than that found by Mayaki et al in Niger [12] and Shittu et al in Nigeria [13] with 0.47% and 0.84% respectively. Our result remains lower than that of Dionne-Odom et al in Cameroon [5] and Nambei et al in CAR [6] who reported 4% and 4.36% respectively.

These variations could be explained on the one hand by risky sexual behavior, marital practices, accessibility to health care, sample size during surveys and donor selection criteria. On the other hand, the differences in sensitivity and specificity of the laboratory tests used by the researchers can also explain this situation, this is all the truer that within the same institution the CNTS, it was reported in the under the activity report a prevalence of 4.14% in 2019 using the strategy of a single screening test (Alere syphilis). The same is true at the Blood Bank in Abéché, in eastern Chad, where a prevalence of 4.9% was reported in 2017 [14].

The majority of our patients were male with 88% of cases against 12% of female (Summary Table).

Our result is similar to that of MBA et al in Gabon [15] who obtained a male predominance of 83.4%. Bassandja et al [10], in their study on the seroprevalence of syphilis among voluntary blood donors in Kisangani in DRC in 2014, found a male predominance of 79.0%. This predominance was also reported by Nambei et al in CAR in 2015 [6] with 95.83%. This could be explained by the predominance of male sex in our blood donation habits and the influence of obstetric factors limiting blood donation in female subjects thus reducing the number of female blood donors.

The most represented age group was that of 24-34 years with 35.78% (Summary Table). The most represented profession was traders 15.44% followed by civil servants 14.48% (Summary Table).

The average age of our donors was 41.5 years old. The most represented age group was 25-34 years old with 35.78%. Our result is close to that obtained by Goita et al in Mali who reported an age group of 26-35 years (38.40%) and to that of N'guessan in Abidjan [16] in 2014 who reported a 25-34 age group with 47.8%. It is also superimposable on that reported by MBA et al in Gabon with an age group of 25-34 years with 45.2% (Summary Table).

This could be explained by the fact that young people are sexually active and run the risk of contracting sexually transmitted diseases, especially syphilis here, more than older people.

According to profession, traders were the most represented with 15.44% followed by civil servants with 14.37% than other socio-professional groups. This result differs from that of Nebie [17], in Burkina Faso who reported a predominance of 13.0% among the military than among students and workers. This could be explained by the strong representativeness of the informal sectors which predominate in professional activities under our skies.

Family replacement donors were the most represented with 94.20% followed by new donors (73%), occasional donors (20.49%) and regular donors (6.57%) respectively (Summary Table).

In our study, family replacement donors were the most represented with 94% (Summary table: 4). This result is similar to that of Goita et al, in Mali, who found 95.47% family donors. These results were superior to that of MBA et al, in Gabon [15] who reported 85.4%.

This is explained by the lack of participation of voluntary donors in our regions despite the efforts made to raise awareness about voluntary blood donation on the one hand and on the other hand by the fact that fraternal links are sacred for donors.

New donors were the most represented with 72%. This result is similar to that of Mayaki et al, in Niger [9] who had reported a predominance in new donors with 58.5%. This could be explained by the lack of loyalty to the blood donation of our populations.

The most represented co-infection was HBV with 6.88 (Summary Table).

In our study, co-infection with HBsAg was the most represented with 6.88% followed by HIV and HCV with 2.45%. This same coinfection was reported with HBsAg-Syphilis by Nambei et al with 12.5% and that of HIV and HCV which were respectively 12.5%, but this prevalence remains higher than that obtained in our study. In 2014, Bessimbaye et al [18] in Chad reported a prevalence of HBsAg at 13.5% in people infected with HIV. This suggests that the prevalence of HBsAg is significantly elevated in blood donors and in the general population.

**Summary Table: The socio-demographic characteristics of donors confirmed by the TPHA test**

Parameter	The socio-demographic characteristics of donors confirmed by the TPHA test							
	Distribution of donors by sex:1		Distribution of donors by age group:2			Distribution by profession:3		
Sex	TPHA Positive (%)	TPHA Negative (%)	Age group	TPHA Positive (%)	TPHA Negative (%)	Profession	TPHA Positive (%)	TPHA Negative (%)
Male	56 (86)	122 (87.14)	18-24	83 (13)	15 (10.71)	Functionary	94 (14.37)	23 (16.43)
Female	91 (14)	18 (13)	25-34	234 (36)	55 (39.29)	Student/pupil	89 (14)	20 (14.29)
Total	654 (100)	140 (100)	35-44	103 (16)	37 (26.43)	Household	47 (7.19)	10 (7.14)
			45-54	106 (16.21)	24 (17.14)	Trader	101 (15.44)	14 (10)
			55-65	128 (19.57)	9 (6.43)	Sex workers	73 (11.16)	19 (13.57)
			Total	654 (100)	140 (100)	cultivator	29 (4.43)	11 (8)
						breeder	20 (3.06)	4 (3)
Donor	TPHA Positive (%)	TPHA Negative (%)	Donor status	TPHA Positive (%)	TPHA Negative (%)	Laborer	71 (11)	14 (10)
FRD	614 (94)	134 (96)	New donor	447 (73)	92 (66)	Driver	61 (9.33)	12 (8.57)
VNRD	40 (6.12)	6 (4)	Regular donor	58 (9)	13 (9.2)	Other	69 (10.55)	13 (9.29)
Total	654 (100)	140 (100)	Occasional donor	149 (23)	35 (25)	Total	654 (100)	140 (100)
			Total	654 (100)	140 (100)			
Co-infection	TPHA Positive (%)	TPHA Negative (%)	TPHA titer	Distribution by TPHA titer: 7				
HIV	16 (2.45)	3 (2.14)	≥ 1/80	Effective (%)				
HBsAg	45 (7)	2 (1.43)	1/160	65 (10)				
HCV	16 (2.45)	2 (1.43)	1/320	214 (33)				
Neither	577 (88.23)	133 (95)	1/640	230 (35,17)				
Total	654 (100)	140 (100)	Total	147 (22,48)				
				654 (100)				

FRD: family replacement donors; VNRD: voluntary non-remunerated donors; % = percentage

The samples with a titer of 1/320 are the most representative with 35.17% (Summary Table).

#### 4. CONCLUSION

Our study, the objective of which is to help improve blood safety, noted a relatively lower proportion of syphilis in donors with the two-test sequential strategy (non-treponemal test and confirmation by treponemal test). This reduces the proportion of syphilis observed so far and allows a gain in blood bag compared to the strategy for a non-treponemal test

#### CONSENT

Informed verbal consent from each syphilitic serology positive donor or their successor to whom we explained the procedures and the importance of the study was obtained for each study participant.

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### ACKNOWLEDGMENTS

We thank family replacement donors and voluntary non-remunerated donors who agreed to donate blood to save human life by participating in this study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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*The peer review history for this paper can be accessed here:*  
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