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ORIGINAL ARTICLE

Performance of the SD Bioline rapid diagnostic test as a good alternative to the detection of human African trypanosomiasis in Cameroon

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Abstract

Background. Case detection is essential for the management of human African trypanosomiasis (HAT), which is caused by *Trypanosoma brucei* gambiense. Prior to parasitological confirmation, routine screening using the card agglutination test for trypanosomiasis (CATT) is essential. Recently, individual rapid diagnostic tests (RDTs) for the serodiagnosis of HAT have been developed.

Objective. The purpose of this study was to evaluate the contribution of SD Bioline HAT to the serological screening of human African trypanosomiasis in Cameroonian foci.

Methods. Between June 2014 and January 2015, blood samples were collected during surveys in the foci of Campo, Yokadouma, and Fontem. The sensitivity (Se) and specificity (Sp) of SD Bioline HAT were determined using the CATT as the gold standard for the detection of specific antibodies against *Trypanosoma brucei* gambiense.

Results. A total of 88 samples were tested: 59.1% (n=52) in Campo, 31.8% (n=28) in Yokadouma, and 9.1% (n=8) in Fontem. There were 61.4% (n=54) males and 38.4% (n=34) females, and the average age was 35.4 19.0 years. In probed foci, the overall seroprevalence was 11.4% (95% confidence interval: 6.3-19.7) with the CATT method and 18.2% (95% confidence interval: 11.5-27.2%) with the SD Bioline HAT RDT method. The SD Bioline HAT's Se and Sp were 80.0% and 89.7%, respectively.

Conclusions. This study demonstrated that the overall performance of the SD Bioline HAT was comparable to that of the CATT, with high specificity in the serological detection of HAT.

INTRODUCTION

Human African trypanosomiasis (HAT), sometimes known as sleeping sickness, is a parasitic disease transmitted by vectors that is endemic in many sub-Saharan African nations.[1] It is caused by a flagellated protozoan of the genus Trypanosoma, which is transmitted naturally to humans by the Tsetse fly. HAT is one of the neglected and lethal tropical illnesses, with its Rhodesian form caused by *Trypanosoma brucei* rhodesiense and its Gambian variant caused by *Trypanosoma brucei* gambiense.[2,3] It is characterized by a clinical presentation that is non-specific and lacks pathognomonic symptoms.[4]

HAT is a cause for concern in intertropical Africa, where it is on the rise.[5] In 2014, the World Health Organization (WHO) recorded 3,797 cases of HAT and in 2015, 2,804 additional cases. The illness was expected to have caused 3,500 fatalities in 2015. Sustained control efforts have reduced the number of new cases, as 992 and 663 cases were reported in 2019 and 2020, respectively, and more than 95% of reported cases were attributable to T. b. gambiense. The Democratic Republic of the Congo (DRC) is the most impacted nation, with over 75% of the gambiense cases notified.[8] The care of the Gambia form is based on the discovery of cases, followed by the administration of the most appropriate medication based on the disease's stage.[9] The typical approach for mass screening is the CATT[10]; however, its employment during active and passive screening is restricted under specific situations. 11 Consequently, new tactics for the battle and management of HAT have been developed.

In several countries, rapid diagnostic tests (RDTs) for the identification of HAT have been developed and reviewed in recent years.[12,13] With the WHO's objective of eradicating HAT by 2030, these tests offer an alternate option for routine screening in endemic areas' health care institutions. For these RDTs to be effectively disseminated, more research must be conducted on their performance. The National Program for the Control of Human African Trypanosomiasis (PNLTHA) is actively studying cases in three foci (Campo, Fontem, and Yokadouma) of HAT in Cameroon.

MATERIALS AND METHODS

Ethical considerations

All necessary precautions were taken to ensure that the rights and freedoms of the participants in the research were respected. In order to conduct the present study, the ethical clearance N°2015/0003 was sought and obtained from the institutional ethics committee for research for human health of the school of health sciences (Yaoundé).

Study design

We conducted a cross-sectional study over a period of nine months, from June 2014 to March 2015. The samples were collected in three foci: Campo in the South, Yokadouma in the East, and Fontem in the Southwest of Cameroon. These foci are geographically propitious zones for hatching Tsetse flies: a temperature of about 25°C and a relative humidity of 80 to 85% and a lot of shade.[14] The participants in the study were the inhabitants of the foci and the refugees from the Central African Republic residing in the Yokadouma camp. However, pregnant women and infants were not included in our study.

Sampling was done by simple random sampling. It consisted in drawing lots directly from individuals in the population of the various foci surveyed.

Participant enrolment process

Our work was conducted during the various surveys organized by the national HAT control program of Cameroon. They were done in several stages, ranging from the census to prior awareness of the target population by the field team. We then approached each participant by presenting the information notice, explaining to him in simple terms the purpose of the study, the interest, the amount of blood to be collected and the samples and results management.

Supplementary information The online version of this article (Tables/Figures) contains supplementary material, which is available to authorized users.

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Anyone who understood and accepted the conditions of the study gave their consent by signing the informed consent form. After this stage, each participant was registered and then taken to perform the different CATT and rapid diagnostic screening tests. At the end, the results were handed to them individually.

Blood sampling consisted in taking from each participant, on the fingertip, about 200 μl of blood. This blood was stored in heparin microcapillary tube and classified on racks numbered from 1 to 10 for the first step of screening. However, for all individuals who tested positive in the first step of screening, whole blood was collected from the elbow in a 4 ml heparinised tube for further testing (CATT dilution and RDTs).

Tests performed

The samples were analysed both in the field and at the HAT laboratory at the Organisation for Coordination of the Control of Endemic Diseases in Central Africa, Yaoundé. Two types of tests were performed for each sample: the agglutination test for trypanosomiasis and the rapid diagnostic test.

Card Agglutination Test for Trypanosomiasis

The Card Agglutination Test for Trypanosomiasis (CATT, Figure 1) is a direct plate agglutination test. It consists in bringing together antigens of trypanosomes, consisting of whole and lyophilized T. b. gambiense; and the whole blood of the person to be examined. It is an interaction between a specific agglutinating antibody and a particular antigen. A drop of reconstituted reagent (about 45 μl) was deposited on the card, a drop of blood (about 25 μl) was added. The mixture was then spread, the card was placed five minutes on a rotary shaker at one revolution per second. The reading was done immediately after the 5 minutes of stirring, with the naked eye with reference to the positive and negative controls. Quantitation was performed on all positive CATT whole blood samples. It consisted in taking 100 μl of whole blood for successive dilutions (1:2, 1:4, 1: 8, 1:16) with 100 μl CATT buffer each time. The titration was done by taking 25 μl of each blood dilution that we put in the test area on the card, before adding a drop of reconstituted reagent. For the rest we proceeded as for the CATT test. This quantification was done in order to determine the positivity threshold of each sample.

The rapid diagnostic test

The rapid diagnostic test SD Bioline HAT is an immunochromatographic test for the rapid and qualitative detection of antibodies named Litat1.3 and Litat1.5, which are specific for T. b. gambiense. The procedure used was according to the manufacturer's instructions (Figure 2). A drop of whole blood was placed in the round window of the test. Subsequently, we added four (4) drops of diluent to this spot, the result was read within 15 minutes of the deposit.

The result was negative when a single-coloured band was observed on "C" in the result window. It was positive when two coloured bands were observed, either on line 1 and control line "C" (positive to Litat 1.3), or on line 2 and control line "C" (positive to Litat 1.5) or again when three coloured bands appeared in the control, 1 and 2 respectively, this means a positive result in Litat 1.3 and 1.5. The result was invalid when the control band "C" did not appear, regardless of the other results observed.

Statistical analysis

Data were analysed using R.3.1.1 software. This analysis was used to calculate prevalence (p), sensitivity (Se) and specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV). A P value of less than 0.05 was significant.

RESULTS

General characteristics

This study involved 88 people recruited as follows in different foci, Campo 59.1% (n = 52), Yokadouma 31.8% (n= 28) and Fontem 9.1% (n = 8). The gender distribution in the foci investigated was 61.4% (n=54) male and 38.4% (n=34) female and the average age was 35.4 $\pm$ 19.0 years, ranging from 5 to 75 years.

HAT seroprevalence

The serological prevalence (Figure 3) of HAT was 36.4% (95% CI: 27.1-46.7) with the whole blood CATT (32/88) and 18.2% (95% CI: 11.5-27.5) with the SD Bioline HAT (16/88). To increase the sen-

sitivity of the CATT, successive dilutions of whole blood were performed. The threshold of suspicion of a case of HAT in Cameroon is represented by a positive CATT from 1:16 of diluted blood (PNLTHA, Cameroon).[15] Based on this national algorithm, our results suggested that the overall seroprevalence with the CATT method was 11.4% (95% CI: 6.3- 9.7) in the probed foci (10/88).

Performance of rapid tests for HAT case detection

The performance of SD Bioline HAT has been calculated taking the CATT as the reference. We observe that, as the dilutions are made, the sensitivities of the RDT increase while the specificities decrease.

The threshold for CATT positivity for blood dilution is 1:16 in Cameroon. At this threshold, the SD Bioline HAT showed a sensitivity of 80.0%, a negative predictive value of 97.22% and the p-value of each was greater than 0.05 indicating a non-significant difference between these values and those of the CATT. At the same threshold, the specificity was 89.7% while the positive predictive value was 50.0% and the p-value of each was less than 0.05, showing a significant difference between the specificity of the CATT and the SD Bioline HAT is statistically. Table 1 shows the values of the intrinsic (Se and Sp) and extrinsic (PPV and NPV) performance indicators of SD Bioline HAT.

DISCUSSION

Our primary objective was to evaluate the contribution of the rapid diagnostic test to the screening of HAT cases in Cameroon for T. b. gambiense. In addition to whole blood, we performed four dilutions of the CATT (1:2, 1:4, 1:8, 1:16) as a reference test. We utilized this RDT for HAT screening because it is currently available and distributed by the institute of tropical medicine. To evaluate this test, samples were collected in Cameroon's Campo, Yokadouma, and Fontem outbreak foci. Campo and Fontem are recognized and active HAT foci in Cameroon, whereas the Yokadouma residence is a suspicious and silent focus.[16,17] The investigation in the latter focus (Yokadouma) tracks the migration of refugees fleeing political instability in the Central African Republic (CAR), the majority of whom are from Nola, an active and well-known HAT focus in the CAR.[18] CATT analyses included 88 individuals, the majority of whom were sampled during the Campo outbreak and the remainder during the Yokadouma and Fontem outbreaks.

The prevalence of serum HAT (CATT-positive on whole blood) was 36% overall. It was reduced to 11.4% by taking into account the dilution of whole blood to 1:16, which corresponds to Cameroon's suspicion threshold for HAT cases requiring surveillance.[15] It should be noted that for HAT screening, the WHO recommends dilutions of positive whole blood CATT tests in order to eliminate the possibility of false positives.

Anyone in Cameroon with a diluted blood CATT 1:16 is considered serologically positive for HAT and is being monitored by PNLTHA. This threshold is set at 1:8 in Chad and the Central African Republic, and 1:4 in the Democratic Republic of the Congo to eliminate false positives.[15]False positives on the CATT test may be caused by cross-reactions following exposure to other pathogens, such as animal trypanosomes: *T. b. brucei* and *T. congolense*.[19] Schistosomiasis, filariasis, and toxoplasmosis have been demonstrated to be capable of agglutinating CATT at low titres.[20,21] During our fieldwork, we also observed the presence of microfilariae in the ganglion fluid of a CATT-positive whole blood sample.

The overall blood test positivity rate was 18.2% when SD Bioline HAT was used as an alternative screening tool. However, at a blood dilution threshold of 1:16, we obtained a prevalence of 11.4% that was closer to the SD Bioline HAT test prevalence of 18.2% than with undiluted blood. These values indicate a close correlation between the detection capabilities of CATT blood 1:16 and SD Bioline HAT.

SD Bioline HAT's sensitivity is measured by its ability to produce positive results in all individuals who have had contact with T.b.gambiense and have blood antibodies against Litat 1.3 and Litat 1.5. All individuals who have never been in contact with T. b. gambiense have exhibited negative responses to the specificity of its capacity. Using the CATT

as a benchmark, these two indicator values of the performance of RDTs to identify serological cases of HAT were calculated.

In contrast to specificity, the sensitivity of this RDT increased as CATT was diluted. At the CATT blood threshold of 1:16, the Se percentage was 80.0% and the Sp percentage was 89.7%. These values indicate that SD Bioline HAT performed satisfactorily when investigating suspected cases of HAT. In 2014, Sternberg *et al.* found Se = 82% and Sp = 97% by evaluating the performance of SD Bioline HAT and two prototype RDTs on 500 samples, including 250 cases and 250 controls from Angola, Central African Republic, and Uganda.[22] In addition, Bisser et al. obtained a sensitivity of 87.8% in the evaluation of the optimization of the same test using 49 confirmed parasitological THA specimens and a specificity of 93-95% after evaluating the SD Bioline HAT and the SD Bioline optimized using 399 control samples in active screening in the DRC.[23] Comparing these RDTs with CATT diluted 1/8 yielded a sensitivity of 89.3 percent as well. The sensitivity of SD Bioline HAT, which was observed to be 80% in this study, was slightly lower than the manufacturer-reported value (98%).

Our small sample size in comparison to theirs could account for the differences. In addition, no parasitological cases were confirmed.

Limitations of the study

This study focuses on HAT disease, which is a rare and neglected tropical disease in Cameroon. The main limitations of this study were limited access to different collection foci, located in remote areas, small sample size, as well as financial limitations for the acquisition of rapid diagnostic tests.

CONCLUSIONS

The study in three HAT foci in Cameroon revealed 18.2% of serological cases with the CATT method and 11.4% with SD Bioline HAT. The performance of SD Bioline HAT RDT compared to the CATT method shows that SD Bioline HAT could be an alternative to be adopted in most HAT foci in Cameroon. Considering that these foci are in remote areas, without appropriate infrastructure and technical platforms for perform CATT.

INFORMATION

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Contribution. ALDW designed the study and wrote the manuscript; AO and SB provided supplies and technical assistance for field collections and laboratory testing; CD, GBDD and CM reviewed the manuscript; USMM and CCN conducted the statistics analysis; HPAA supervised the study and reviewed the manuscript; HPAA and NFD coordinated the study.

Conflict of interest. the authors declare no potential conflict of interest.

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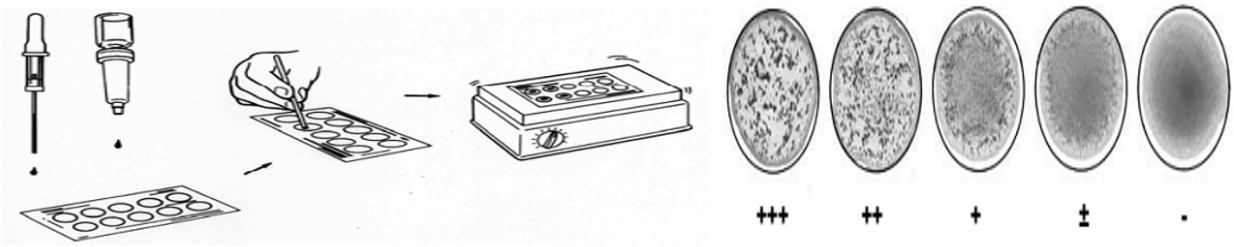

+++ Positive (Very strong agglutination)
++ Positive (strong agglutination)
+ Positive (Moderate agglutination)
± Weakly positive
- Negative (no agglutination)

FIGURE 1: Stages ofthe CATT (Test Guide CATT/T.b.gambiense Institute of Tropical Medicine) CATT: Card Agglutination Test for Trypanosomiasis

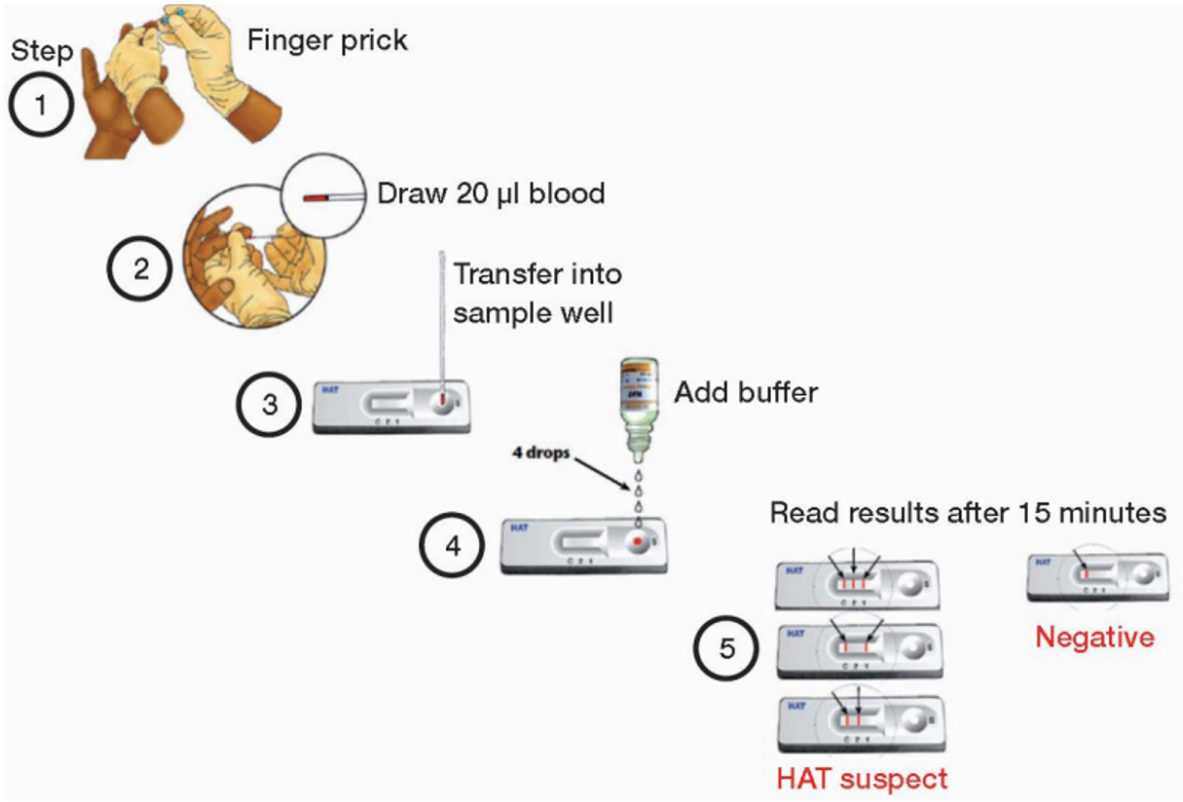

FIGURE 2: Procedure of the DS Bioline HAT test (Lumbala et al.; 2013). In total, the CATTplate agglutination test and the SD Bioline HAT were performed on 88 samples.These two tests made it possible to detect specific antibodies LiTat 1.3 andLiTat 1.5 from T. b. Gambiense in allprospects.

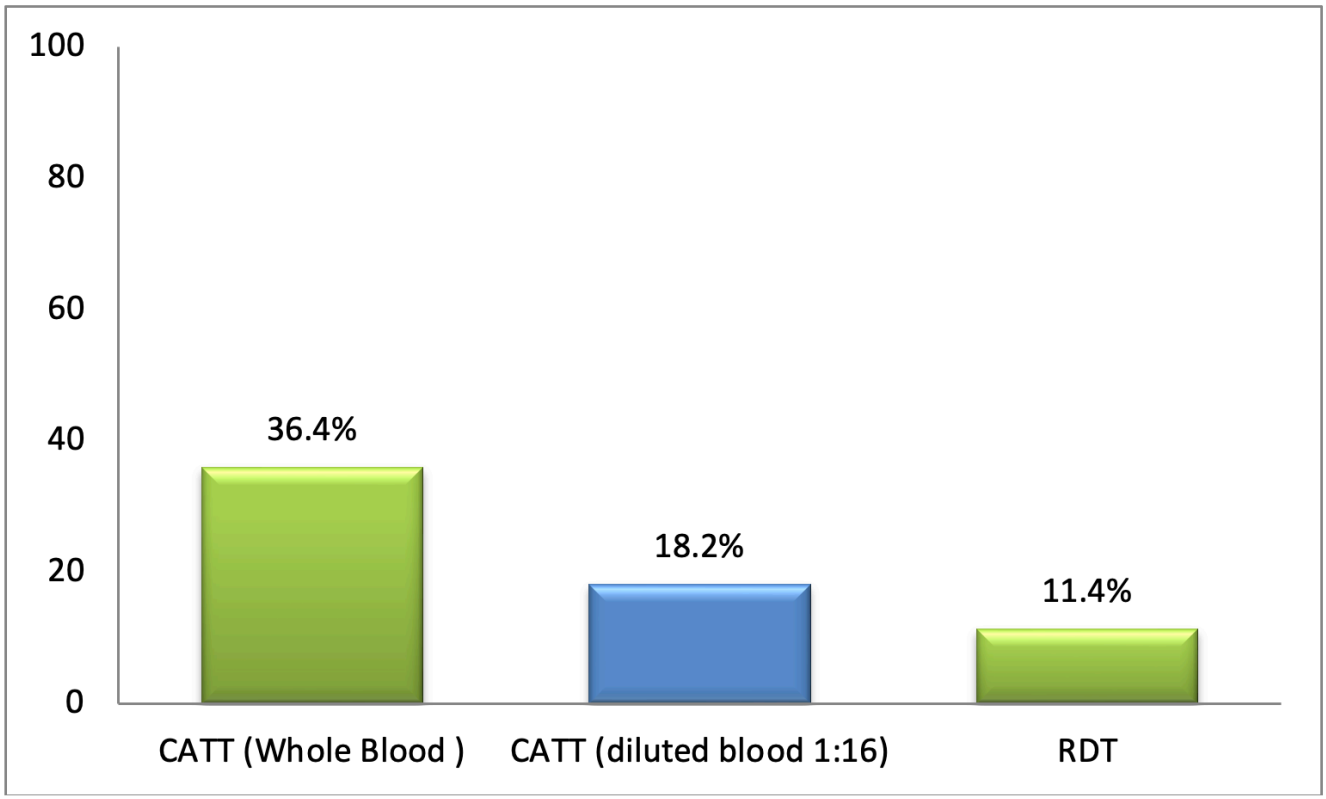

FIGURE 3: Serological prevalence. CATT ST: Card Agglutination Test for Trypanosomiasis with Whole Blood; CATT 1:16: Card Agglutination Test for Trypanosomiasis diluted 1:16; RDT: Rapid Diagnostic Test

TABLE 1: Descriptive statistics of the performance values of SD Bioline HAT.

SD Bioline HAT	CATT (Whole blood)	CATT (Diluted blood 1:4)	CATT (Diluted blood 1:8)	CATT (Diluted blood 1:16)
Se (%)	**50.0**	**53.6**	**60.0**	**80.0**
P Value	0.0002	0.0009	0.0133	0.4795
Sp (%)	**100**	**98.3**	**94.1**	**89.7**
P Value	1	1	0,1336	0.0133
PPV (%)	**100**	**94.0**	**75.0**	**50.0**
P Value	1	1	0.1336	0.01333
NPV (%)	**77.8**	**81.9**	**88.9**	**97.2**
P Value	0.0002	0.0009	0.0133	0.4795

%: percentage, Se: Sensitivity, Sp: Specificity, WB: Whole Blood, PPV: Positive Predictive Value, NPV: Negative Predictive Value. P value < 0.05 is significant.