

# BMJ Open Diagnostic performance of the Hightop Rapid Diagnostic Test for the detection of anti-*Treponema pallidum* antibodies in Cameroon: a laboratory-based cross-sectional and comparative study

Christian Taheu Ngounouh <sup>1,2</sup>, Philippe Salomon Nguwoh,<sup>2</sup> Joseph Fokam,<sup>3</sup> René Ghislain Essomba,<sup>4</sup> Ibrahima Halilou,<sup>2</sup> Amina Al-Mayé Bit Younouss,<sup>2</sup> Nafissatou Ibnou Moussa,<sup>2</sup> Emile Yuosembom,<sup>2</sup> Solange Pepa,<sup>2</sup> Marcel Mbarga Foe,<sup>2</sup> Emmanuel Orock Eta,<sup>2</sup> Guianni Mpiwouo Panyere,<sup>2</sup> Corine Madie Tamo,<sup>5</sup> Jérôme Ngantchui Tchuisseu,<sup>2</sup> Aboubakar Moussa,<sup>6</sup> Elie Zotie,<sup>7</sup> John Francois Ndombol Tembten,<sup>8</sup> Lydie Nyatte,<sup>9</sup> Victor Fondoh,<sup>10</sup> Constance Nyanda Nyeng,<sup>11</sup> Youssoufa Taoufick,<sup>12</sup> Dodo Nyako Balkissou,<sup>12</sup> Blaise Akenji Mboringong,<sup>2</sup> Hamsatou Hadja,<sup>2</sup> Marie Claire Okomo Assoumou<sup>4</sup>

**To cite:** Taheu Ngounouh C, Nguwoh PS, Fokam J, *et al*. Diagnostic performance of the Hightop Rapid Diagnostic Test for the detection of anti-*Treponema pallidum* antibodies in Cameroon: a laboratory-based cross-sectional and comparative study. *BMJ Open* 2025;15:e093330. doi:10.1136/bmjopen-2024-093330

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<https://doi.org/10.1136/bmjopen-2024-093330>).

Received 04 September 2024  
Accepted 11 August 2025



© Author(s) (or their employer(s)) 2025. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ Group.

For numbered affiliations see end of article.

## Correspondence to

Christian Taheu Ngounouh; taheuchristian@gmail.com

## ABSTRACT

**Objective** The study was conducted to assess the diagnostic performance of the Hightop Syphilis Rapid Diagnostic Test (RDT) in comparison with the ELISA test used as a reference method.

**Design** A laboratory-based cross-sectional and comparative study was conducted to assess the diagnostic performance of the Hightop Syphilis RDT.

**Setting** Blood samples obtained from adult participants in eight health facilities were analysed at the National Public Health Laboratory (NPHL), Ministry of Public Health, Yaounde, Cameroon.

**Participants** From 29 April to 25 August 2023, 583 adult participants of both sexes (aged  $\geq 21$  years), including both syphilis positive and syphilis negative, were recruited consecutively in eight health facilities in eight regions of Cameroon.

**Outcome measures** Blood samples were screened for the detection of anti-*Treponema pallidum* antibodies using the One Step Rapid Test (Qingdao Hightop Biotech), a non-treponemal test and ELISA (Biorex Diagnostics, UK), a treponemal test used as a reference method. Diagnostic performance of the Syphilis RDT was analysed using Epi Info V.7 and validated through online statistical tools such as StatPages, GraphPad, QuickCalcs and MedCalc software.

**Results** Of the 583 samples tested, the Hightop Syphilis RDT revealed a sensitivity of 84.6% (95% CI: 74.8% to 91.1%) and specificity of 98.5% (95% CI: 97.5% to 99.1%). The positive predictive value (PPV) and negative predictive value (NPV) were 84.6% (95% CI: 74.8% to 91.1%) and 98.5% (95% CI: 97.5% to 99.1%), respectively. Regarding the stratification of diagnostic performance by clinical stage, the test showed a sensitivity of 100.0% (95% CI: 71.51% to 100.0%) and specificity of 99.06% (95% CI: 94.86% to 99.98%). The PPV and NPV

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The study involved a large sample of blood drawn from syphilis-positive and syphilis-negative individuals in health facilities in 8 of the 10 regions of Cameroon.
- ⇒ The Hightop Syphilis Rapid Diagnostic Test exhibits excellent diagnostic capabilities in both symptomatic and asymptomatic individuals.
- ⇒ The study does not differentiate between syphilis stages (primary, secondary and tertiary disease) due to the design.

were 91.67% (95% CI: 61.00% to 98.72%) and 100.0% (95% CI: 96.55% to 100.0%), respectively, in symptomatic individuals. Among asymptomatic individuals, sensitivity was 97.56% (95% CI: 87.14% to 99.94%) and specificity was 100.0% (95% CI: 99.14% to 100.0%). The PPV and NPV were 100.0% (95% CI: 91.19% to 100.0%) and 99.77% (95% CI: 98.40% to 99.97%), respectively.

**Conclusions** The Hightop Syphilis RDT demonstrated adequate diagnostic performance, particularly among symptomatic individuals, supporting its utility as a reliable tool for syphilis detection in clinical settings.

## INTRODUCTION

Syphilis is a sexually transmitted infection (STI) caused by *Treponema pallidum*, a pathogenic bacterium belonging to the genus *Treponema* within the phylum Spirochaetes.<sup>1 2</sup> Syphilis remains a global public health priority, with an estimated 17.7 million individuals aged 15–49 years infected in 2012 and approximately 5.6 million new cases



occurring annually worldwide.<sup>3</sup> In 2023, the Centers for Disease Control and Prevention (CDC) recorded over 2.4 million STI cases, including 209 000 of syphilis, 600 000 of gonorrhoea and 1.6 million of chlamydia.<sup>4</sup> Alarmingly, 3882 congenital syphilis cases were documented, resulting in 279 stillbirths and neonatal deaths.<sup>4</sup> Sub-Saharan Africa bears the highest global burden of syphilis, with more than 60% of newly diagnosed cases worldwide each year.<sup>3,5</sup> *T. pallidum* is transmitted through several routes, including transfusion of contaminated blood, unprotected sexual contact both heterosexual (men who have sex with women) and homosexual (men who have sex with men) as well as vertical transmission from mother to child during pregnancy and breastfeeding.<sup>1 6 7</sup> Syphilis progresses through four clinical stages, of which primary, secondary and early latent stages are infectious. Untreated, primary syphilis can progress towards the secondary, latent and tertiary stages of the disease.<sup>8</sup>

Serological testing remains the keystone of syphilis diagnosis, serving as the primary method for detecting asymptomatic infections and for confirming clinical suspicion in symptomatic patients.<sup>2</sup> Accordingly, syphilis diagnosis and staging require the combined use of two types of serological tests: non-treponemal tests (NTTs) and treponemal tests (TTs).<sup>2</sup> NTTs, such as the rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) method, lack specificity due to cross-reactivity with antibodies produced in various other pathological conditions, which can result in false-positive test outcomes.<sup>2 9</sup> When one or both NTTs were positive results, confirmation is performed using TTs such as the fluorescent treponemal antibody absorption (FTA-ABS), *T. pallidum* particle agglutination (TPPA) or enzyme immunoassay.<sup>10</sup>

In Cameroon, the conventional syphilis screening algorithm recommends initial testing with an NTT (RPR or VDRL), followed by confirmation using a TT if the initial test is reactive.<sup>11</sup> A study conducted by Solaimalai *et al*<sup>12</sup> in India reported that the ELISA showed a sensitivity of 98% and specificity of 97.5%. In contrast, the VDRL test demonstrated a sensitivity of 71.6% and specificity of 89.5%, while the RPR test showed a sensitivity of 73.5% and specificity of 90.5%. Furthermore, ELISA demonstrated excellent concordance with the *T. pallidum* haemagglutination assay (TPHA), reflected by a kappa coefficient of 0.9, whereas VDRL and RPR showed moderate concordance with TPHA (kappa=0.6).<sup>12</sup> Several studies worldwide have evaluated the diagnostic performance of the syphilis rapid diagnostic tests (RDTs) across diverse population groups.<sup>13–15</sup> To date, there is a limited number of studies globally that have evaluated the diagnostic performance of the Hightop Syphilis RDT relative to the reference ELISA, particularly in Cameroon.

## Rationale

Syphilis remains a major public health concern, particularly in resource-limited settings where diagnostic capacity

is constrained. While RDTs offer a promising alternative for early detection, their utility must be validated in real-world contexts. This study aimed to assess the diagnostic performance of the Hightop Syphilis RDT compared with ELISA, with a view to informing syphilis screening strategies in Cameroon and similar settings.

## Study objective

This current study was conducted to assess the diagnostic performance of the Hightop Syphilis RDT in comparison with the ELISA used as a reference method.

## MATERIALS AND METHODS

### Study design and setting

This laboratory-based cross-sectional study was designed to assess the laboratory performance of the Hightop Syphilis RDT compared with ELISA used as a reference method for the diagnosis of syphilis in Cameroon. The sample (serum and plasma) was collected in eight regions of the country and performed at the National Public Health Laboratory (NPHL).

### Participant and data collection

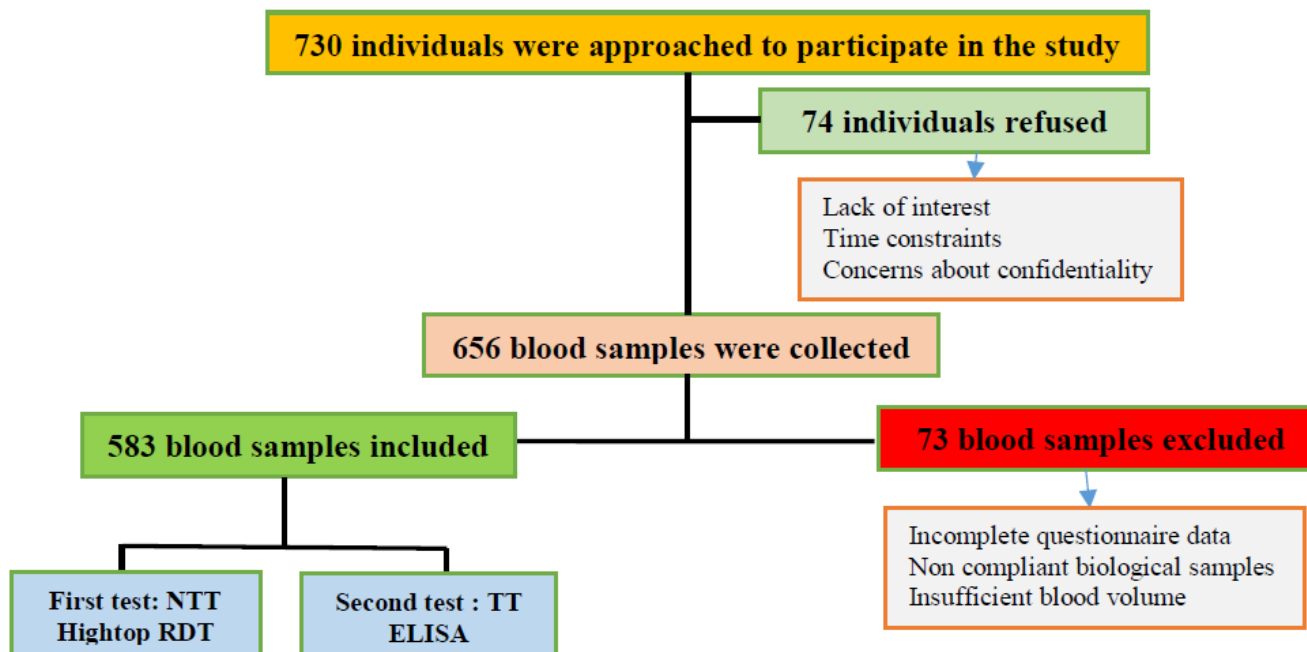
From 29 April to 25 August 2023, a total of 583 adult participants (aged  $\geq 21$  years) of both sexes were consecutively enrolled in eight healthcare facilities across the country. Prior to participation, all individuals were provided with a written information note outlining the study's objectives, procedures, potential risks and benefits. Written informed consent was obtained from each participant. Demographic and clinical data were collected through structured, face-to-face interviews using a coded questionnaire (online supplemental file S1). Venous blood samples were collected using sterile needles and vacuum collection systems. Specimens were drawn into dry tubes for serum and EDTA tubes for plasma. Blood samples were centrifuged at 1500 rpm for 5 min; serum and plasma were aliquoted into colour-coded cryovials (red for serum, blue for plasma). Samples were transported under cold chain conditions in ice-packed coolers by ground to the NPHL. On receipt, samples were stored at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  until laboratory analysis. Each specimen was labelled with a unique identifier and accompanied by the participant's coded questionnaire data to maintain data integrity and traceability.

### Inclusion and exclusion criteria

The study included adult participants aged 21 years and above, of both sexes who voluntarily agreed to participate. Participants with incomplete questionnaire data, insufficient and non-compliant biological samples were excluded (figure 1).

### Data sources and variables

The present study used primary data collected from participants attending routine consultation in health facilities across eight regions of the country following a face-to-face interview. Quantitative variables such as age,



**Figure 1** A chart flow diagram showing the process of participant recruitment. NTT, non-treponemal test; RDT, Rapid Diagnostic Test; TT, treponemal test.

qualitative variables such as gender and laboratory results were collected and recorded in a coded questionnaire. The prevalence of syphilis (qualitative variable) was measured using Hightop Syphilis RDT and confirmed by ELISA.

#### Sampling method and sample size determination

The participants were enrolled using a non-probabilistic sampling method. The sample size was calculated using the Kish and Leslie formula for cross-sectional studies:  $n = Z^2 \times p \times q / m^2$ ,<sup>16</sup> where  $n$  is the required sample size,  $Z$  is the standard normal deviate corresponding to a 95% CI ( $Z=1.96$ ),  $p$  is the estimated prevalence (50%),  $q=1-p$  and  $m$  is the error (5%).

#### Laboratory testing

Blood samples were tested using the One Step Rapid Test (Qingdao Hightop Biotech; Reference: H133; Lot: TP1220904; date of manufacture: 15 September 2022; expiry date: 14 September 2024). The Hightop test is a lateral flow chromatographic immunoassay based on a sandwich format, designed for the qualitative detection of antibodies to TP in serum, plasma or whole blood. Test results were interpreted in accordance with the manufacturer's guidelines. A reactive (positive) result was defined by the presence of two distinct red lines: one in the control (C) and one in the test (T) zone. A non-reactive (negative) result was indicated by a single red line in the control (C) zone, with no line in the test (T) zone. Results were considered invalid if (1) a red line appeared in the test (T) zone without a corresponding line in the control (C) zone or (2) no red lines appeared in either zone within the recommended reading window of 15–20 min. The diagnostic performance of the Hightop Syphilis RDT

was assessed by comparison with a qualitative sandwich-format ELISA, used as the reference method. The ELISA was manufactured by Biorex Diagnostics (Antrim Technology Park, BT41 1QS, UK), certified under ISO 13485 (Reference: BXE0995A; Lot No.: TP-2210-1; date of manufacture: October 2022; expiry date: June 2024). All procedures were conducted in accordance with the respective manufacturers' instructions. The ELISA method was selected as the reference standard due to its high sensitivity and reliability in detecting anti-*Treponema pallidum* antibodies associated with syphilis infection.<sup>11 17</sup>

#### Statistical analysis

The data were entered into Microsoft Excel V.2016 and subsequently converted to Excel V.97-2003 sheet. The dataset was imported into Epi Info V.7 (CDC, Atlanta, USA) for statistical analysis. Descriptive statistics, including means, SD, frequencies and percentages, were calculated to summarise demographic and clinical characteristics of the study population. Diagnostic performance metrics including sensitivity, specificity, positive

**Table 1** Distribution of the Hightop Syphilis RDT results based on reference method

Reference method using ELISA			
Hightop syphilis RDT	Reactive	Non-reactive	Total
Reactive	44 (TP)	8 (FP)	52
Non-reactive	8 (FN)	523 (TN)	531
Total	52	531	583

FN, false negative; FP, false positive; RDT, Rapid Diagnostic Test; TN, true negative; TP, true positive.

predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratios (LR<sup>+</sup> and LR<sup>-</sup>) and Cohen's kappa coefficient ( $\kappa$ ) were calculated using Epi Info V.7 and validated through online statistical tools such as StatPages, GraphPad, QuickCalcs and MedCalc software. 95% confidence interval (95%CI) were reported for all diagnostic indices. Furthermore, the receiver operating characteristic (ROC) curve was constructed using MedCalc<sup>18</sup> to evaluate the overall discriminative ability of the Hightop Syphilis RDT. The area under the curve (AUC) was computed to quantify test accuracy. ROC curves were also stratified by clinical presentation (symptomatic vs asymptomatic) to assess performance variation across subgroups.

## RESULTS

### Demographic characteristics of participants

The study enrolled 583 participants aged between 21 and 81 years (mean: 38.96, SD  $\pm$  12.46 years). The majority was female sex (70.33%), aged 21–30 years (30.87%), had attained secondary education (37.39%), was married or cohabiting (51.80%), resided in urban settings (75.30%), was asymptomatic at presentation (79.93%) and identified as Christian (61.92%).

### Distribution of the Hightop Syphilis RDT results based on reference method

Out of the 583 blood samples analysed, the reference method identified 52 reactive and 531 non-reactive cases. The Hightop Syphilis RDT correctly detected 44 as true positives and 523 as true negatives. However, it yielded 8 false-reactive results (false positives) and missed 8 ELISA-reactive cases (false negatives) (table 1).

### Diagnostic performance of the Hightop Syphilis RDT based on the reference method

The diagnostic performance of the Hightop Syphilis RDT, as compared with the reference ELISA method, demonstrated a sensitivity of 84.6% (95% CI: 74.8% to 91.1%) and a specificity of 98.5% (95% CI: 97.5% to 99.1%). The PPV and NPV were also 84.6% (95% CI: 74.8% to 91.1%) and 98.5% (95% CI: 97.5% to 99.1%), respectively. The positive likelihood ratio (LR<sup>+</sup>) was estimated between 30.26 and 105.16, while the negative likelihood ratio (LR<sup>-</sup>) was 0.1562 (95% CI: 0.089 to 0.259). The overall diagnostic accuracy and Cohen's kappa coefficient were similar with 83.1% (95% CI: 72.3% to 90.3%) (table 2).

### An ROC curve of the Hightop Syphilis RDT

The ROC curve illustrates the balance between sensitivity (true positive rate) and 1–specificity (false positive rate). In this study, the Hightop Syphilis RDT exhibited a sensitivity of 84.6% and a specificity of 98.5%, positioning its performance point well above the line of no discrimination. The AUC was 0.92, indicating high overall diagnostic accuracy (figure 2).

**Table 2** Diagnostic performance of the Hightop Syphilis RDT based on the reference method

	Value	95% CI
Sensitivity	84.6%	74.8% to 91.1%
Specificity	98.5%	97.5% to 99.1%
PPV	84.6%	74.8% to 91.1%
NPV	98.5%	97.5% to 99.1%
LR+	56.41	30.26 to 105.16
LR-	0.1562	0.089 to 0.259
Accuracy	83.1%	72.3% to 90.3%
Kappa	83.1%	72.3% to 90.3%

LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RDT, Rapid Diagnostic Test.

### Distribution of the results of both tests according to the clinical stage

Among the 583 participants enrolled, 20.07% (117/583) were symptomatic, while 79.93% (466/583) were asymptomatic. In the symptomatic group, the Hightop Syphilis RDT identified 100% (11/11) of true positive cases, with no false negatives and a single false positive result (0.94%, 1/106), yielding 99.06% (105/106) true negatives. Among asymptomatic individuals, the test demonstrated a true positive rate of 97.56% (40/41), with one false negative (2.44%) and no false positives, resulting in 100% (425/425) specificity (table 3).

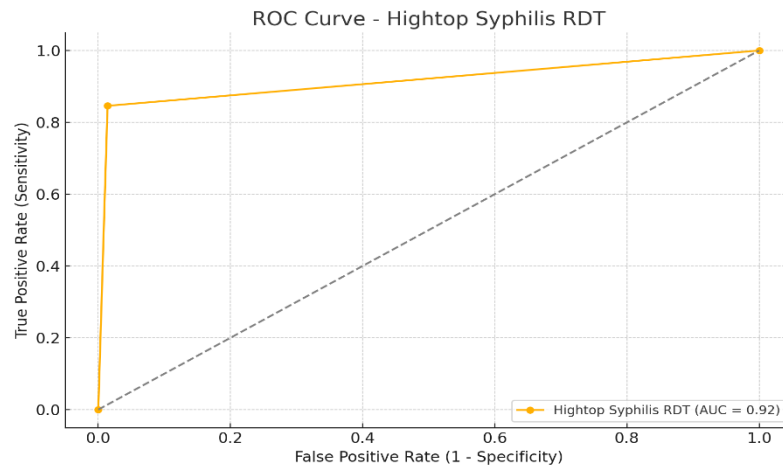
### Diagnostic performance of the Hightop Syphilis RDT based on the reference method according to the clinical stage

In symptomatic individuals, the Hightop Syphilis RDT revealed a sensitivity of 100.0% (95% CI: 71.51% to 100.0%) and specificity of 99.06% (95% CI: 94.86% to 99.98%). The PPV and NPV were 91.67% (95% CI: 61.00% to 98.72%) and 100.0% (95% CI: 96.55% to 100.0%), respectively. The test showed an LR<sup>+</sup> of 106.00 (95% CI: 15.07 to 745.59), an LR<sup>-</sup> of 0.00, an overall accuracy of 99.15% (95% CI: 95.33% to 99.98%) and a Cohen's kappa coefficient of 95.20% (95% CI: 68.0% to 96.0%) (table 4).

Among asymptomatic individuals, sensitivity was 97.56% (95% CI: 87.14% to 99.94%) and specificity was 100.0% (95% CI: 99.14% to 100.0%). The PPV and NPV were 100.0% (95% CI: 91.19% to 100.0%) and 99.77% (95% CI: 98.40% to 99.97%), respectively. LR<sup>+</sup> was not applicable due to the absence of false positives, while LR<sup>-</sup> was 0.02 (95% CI: 0.00 to 0.17). The test showed an accuracy of 99.79% (95% CI: 98.81% to 99.99%) and a kappa value of 98.6% (95% CI: 90.50% to 99.10%) (table 4).

### ROC curve of the Hightop Syphilis RDT according to clinical stage

The ROC curve illustrates the diagnostic performance of the Hightop Syphilis RDT in both symptomatic and asymptomatic individuals. The blue line represents the



**Figure 2** An ROC curve connects coordinate points with 1–specificity (= false positive rate) as the x-axis and sensitivity as the y-axis at all cut-off values measured from the test results. AUC, area under the curve; RDT, Rapid Diagnostic Test; ROC, receiver operating characteristic.

symptomatic group, with an AUC of approximately 1.00, indicating perfect diagnostic accuracy. The green line represents the asymptomatic group, also with an AUC near 1.00, demonstrating excellent performance even in the absence of symptoms. The diagonal grey dashed line represents a non-informative test (AUC=0.5) (figure 3).

## DISCUSSION

The study was conducted to assess the laboratory performance of the Hightop RDT for syphilis diagnosis in Cameroon. A comparison was made between the Hightop Syphilis RDT and ELISA, used as a reference test. A total of 583 venous blood samples were obtained from adult participants attending eight healthcare facilities. The Hightop Syphilis RDT demonstrated a sensitivity of 84.6% (95% CI: 74.8% to 91.1%), indicating a strong capacity to detect true positive cases (table 2). Sensitivity is a key metric in assessing a diagnostic tool's ability to

correctly identify infected individuals, particularly in screening settings where early detection is critical for timely treatment and interruption of transmission. The suboptimal sensitivity observed may be partly explained by the predominance of asymptomatic individuals in the study population, likely associated with lower antibody titres, thus reducing test detectability. Nonetheless, the sensitivity corroborates with findings from previous studies, such as those by Kamolrattana *et al*,<sup>15</sup> Lodiogon *et al*,<sup>19</sup> and Van Den Heuvel *et al*,<sup>20</sup> which reported sensitivities ranging from 85% to 87% for similar RDTs<sup>15 19 20</sup> who reported a sensitivity of 85.7% (95%CI 63.7% to 97.0%), 86.4% (95% CI: 65.1% to 97.1%) and 87%, respectively, using Bioline HIV/Syphilis Dual test and Determine Syphilis RDT. In contrast, our findings diverge from those reported by Kamolrattana *et al* and Van Den Heuvel *et al*, who documented lower sensitivities of 73.5% and 66.7% (95% CI: 43.0% to 85.4%), respectively, when evaluating the Multiplo TP/HIV Duo Test and the Bioline Syphilis 3.0 assay.<sup>15 20</sup> The specificity of the Hightop Syphilis RDT was 98.5% (95% CI: 97.5% to 99.1%) (table 2). Specificity measures the ability of the test to correctly identify individuals without the disease, minimising false positives. The high specificity of the Hightop Syphilis RDT reinforces its capability to accurately identify those without syphilis, which is crucial for reducing unnecessary follow-ups and anxiety for patients. The specificity obtained in our study corroborates the study conducted by Zhang *et al* who reported a specificity of 98% (95% CI: 96% to 99%) across 17 studies in a meta-analyses using treponemal component.<sup>21</sup> Meanwhile, our specificity is lower compared with the study performed by Van Den Heuvel *et al*<sup>20</sup> who reported specificities ranging from 99.0% to 100% using Dual test Multiplo and the Bioline. The difference can be explained by the sample size, the study population and the type of test used (One-step RDT vs Dual test).

**Table 3** Distribution of the results of both tests according to the clinical stage

Hightop Syphilis RDT	Reference method using ELISA		
	Reactive	Non-reactive	Total
Symptomatic			
Reactive	11 (TP)	1 (FP)	12
Non-reactive	0 (FN)	105 (TN)	105
Total	11	106	117
Asymptomatic			
Reactive	40 (TP)	0 (FP)	40
Non-reactive	1 (FN)	425 (TN)	426
Total	41	425	466

FN, false negative; FP, false positive; RDT, Rapid Diagnostic Test; TN, true negative; TP, true positive.

**Table 4** Diagnostic performance of the Hightop Syphilis RDT based on the reference method according to the clinical stage

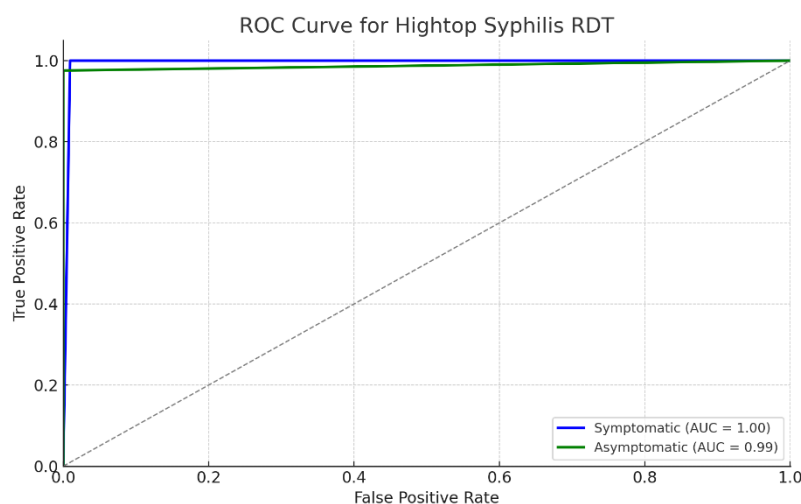
	Clinical stage			
	Symptomatic		Asymptomatic	
	Value	95% CI	Value	95% CI
Sensitivity	100.0%	71.51% to 100.0%	97.56%	87.14% to 99.94%
Specificity	99.06%	94.86% to 99.98%	100.0%	99.14% to 100.0%
PPV	91.67%	61.00% to 98.72%	100.0%	91.19% to 100.0%
NPV	100.0%	96.55% to 100.0%	99.77%	98.40% to 99.97%
LR+	106.00	15.07 to 745.59	Na	Na
LR-	0.00	0.0	0.02	0.00 to 0.17
Accuracy	99.15%	95.33% to 99.98%	99.79%	98.81% to 99.99%
Kappa	95.20%	68.0% to 96.0%	98.6%	90.50% to 99.10%

LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RDT, Rapid Diagnostic Test.

In this current study, the sensitivity and PPV of the Hightop Syphilis RDT were similar, with 84.6% (95% CI: 74.8% to 91.1%) (table 2). PPV reflects the proportion of true positives among all positive test results, indicating the likelihood that a positive result correctly identifies the disease. In the same vein, the specificity and the NPV of the Hightop syphilis test were similar with 98.5% (95% CI: 97.5% to 99.1%) (table 2). NPV measures the proportion of true negatives among all negative test results, highlighting the ability of the test to accurately rule out the disease. A similar performance was reported by the study conducted by Shimelis and Tadesse<sup>22</sup> reported for the diagnosis of syphilis, a PPV and NPV of 95.4% (95% CI: 89.3% to 98.5%) and 98% (95% CI: 93.4% to 99.7%), respectively, using HIV/Syphilis Dual test. Moreover, our findings reported that the accuracy and kappa (k) were similar with 83.1% (95% CI: 72.3% to 90.3%) (table 2). Accuracy provides a broad measure of overall

test performance, integrating both sensitivity and specificity, while kappa measures the agreement between the test results and the actual disease status, accounting for chance agreement. Our findings demonstrate the excellent concordance between Hightop Syphilis RDT and ELISA. The Cohen's Kappa ( $\kappa$ ) obtained in this study was lower compared with the study done by Lodiongo *et al* who reported a k of 0.92 (95% CI: 0.980 to 0.999) using Dual test.<sup>19</sup>

Stratified analysis showed that the Hightop Syphilis RDT performed exceptionally in symptomatic individuals, with a sensitivity of 100%, specificity of 99.06% and overall accuracy of 99.15% (table 3). These results are consistent with findings from a Brazilian study evaluating a treponemal RDT, which reported sensitivities of 75–100% depending on VDRL titres, with 91.9% sensitivity in VDRL-reactive samples and specificities up to 100%, including among HIV-positive individuals.<sup>23</sup> Notably,

**Figure 3** The ROC curve showing the diagnostic performance of the Hightop Syphilis RDT in both symptomatic and asymptomatic individuals. AUC, area under the curve; RDT, Rapid Diagnostic Test; ROC, receiver operating characteristic.

pooled sensitivity exceeded 98% in high-titre cases, a trend similarly observed in our symptomatic cohort, indicating that antibody detection is more effective during active syphilis infection.<sup>21</sup> Furthermore, meta-analyses of treponemal RDTs report pooled sensitivity and specificity of approximately 93% and 98%, respectively, while non-treponemal components demonstrate sensitivity around 90% and specificity near 97%.<sup>21</sup>

The Hightop Syphilis RDT showed excellent diagnostic performance among asymptomatic individuals, with a sensitivity of 97.6% (95% CI: 87.1% to 99.9%) and a specificity of 100.0% (95% CI: 99.1% to 100.0%). Predictive values were also high, with a PPV of 100.0% (95% CI: 91.2% to 100.0%) and an NPV of 99.8% (95% CI: 98.4% to 99.9%). The overall accuracy rate was 99.8%, and the Cohen's kappa value ( $\kappa=0.986$ ; 95% CI: 0.905 to 0.991) reflected almost perfect agreement with the ELISA reference standard (table 3). These findings are consistent with pooled meta-analyses showing that treponemal RDTs maintain high specificity (approximately 98–99%) and moderate-to-high sensitivity (85–95%) among asymptomatic individuals under laboratory conditions. A recent WHO-supported systematic review similarly reported that dual-component syphilis RDTs achieved pooled sensitivity and specificity of 86% and 97% for treponemal targets, and 80% and 96% for non-treponemal components, respectively.<sup>21</sup>

In this study, the Hightop Syphilis RDT showed a sensitivity of 84.6% and specificity of 98.5%, with an area under the ROC curve (AUC) of 0.92, indicating high overall diagnostic accuracy and excellent discriminatory ability. The ROC curve analysis, stratified by clinical stage, revealed AUCs nearing 1.0 in both symptomatic and asymptomatic individuals, indicating that the test reliably distinguishes between infected and non-infected cases across different clinical stages. These findings are consistent with data from a systematic review and meta-analysis conducted by Zhang *et al*, which reported pooled AUCs between 0.90 and 0.95 for treponemal RDTs, confirming their robust diagnostic performance in diverse populations.<sup>21</sup> The near-perfect AUC values observed in symptomatic individuals are likely associated with higher antibody titres in active syphilis, a trend similarly reported in other RDT evaluations such as those by Arai *et al*, where sensitivity improved significantly with increasing VDRL titres.<sup>23</sup>

### Study limitations

The study has several limitations. First, the cross-sectional design limited the ability to track the natural course of syphilis infection or antibody dynamics over time. As a result, diagnostic performance could not be evaluated across different stages of infection (primary, secondary, latent or tertiary). Second, clinical staging of syphilis was based solely on self-reported symptoms and physical examination without laboratory confirmation tests such as dark-field microscopy or PCR, introducing a risk of classification bias. Third, the sample included a high proportion of asymptomatic individuals, which may have

led to an underestimation of sensitivity, as low antibody titres are more common in early or latent stages of infection. Furthermore, using ELISA alone as the reference standard without confirmatory tests of the other TT such as FTA-ABS or TPPA may have led to an overestimation of prevalence and diagnostic accuracy. Finally, the lack of verification for recent antibiotic exposure may have resulted in false-negative results, and recruitment from selected health facilities introduces potential selection bias, thereby limiting the generalisability.

### Conclusion

The Hightop Syphilis RDT shows strong, satisfactory diagnostic performance, with high specificity and strong concordance with ELISA results. Its ease of use, rapid turnaround time and robustness in both symptomatic and asymptomatic populations support its application as a valuable screening tool in resource-limited settings. These findings contribute evidence for the integration of this test into national syphilis screening strategies. Further research is warranted to evaluate its longitudinal performance and applicability across different stages of infection.

### Author affiliations

<sup>1</sup>Higher Institute of Sciences and Techniques Applied to Health, Yaounde, Cameroon

<sup>2</sup>National Public Health Laboratory, Yaounde, Cameroon

<sup>3</sup>Faculty of Health Sciences, University of Buea, Chantal BIYA International Reference Centre for research on HIV/AIDS prevention and Management, Yaounde, Cameroon

<sup>4</sup>Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Yaounde, Cameroon

<sup>5</sup>Laboratory Service, National Insurance Fund Welfare Hospital, Yaounde, Cameroon

<sup>6</sup>Maroua Regional Hospital, Ministry of Public Health, Maroua, Cameroon

<sup>7</sup>Bertoua Regional Hospital, Ministry of Public Health, Bertoua, Cameroon

<sup>8</sup>Nkongsamba Regional Hospital, Ministry of Public Health, Nkongsamba, Cameroon

<sup>9</sup>Ebolowa Regional Hospital, Ministry of Public Health, Ebolowa, Cameroon

<sup>10</sup>Regional Hospital Bamenda Laboratory, Bamenda, North West Region, Cameroon

<sup>11</sup>Limbe Regional Hospital, Ministry of Public Health, Limbe, Cameroon

<sup>12</sup>Garoua Regional Hospital, Ministry of Public Health, Garoua, Cameroon

**Acknowledgements** The authors would like to express their thanks to the individuals for their participation in this study. We also like to express our sincere gratitude to the laboratory technicians of the eight regions for the blood samples collection. Moreover, we also like to acknowledge the technical staff of NPHL for the technical support in blood analysis, particularly to Mme Cressence Fouda.

**Contributors** The study was conceptualised and designed by CTN, PSN, JF, IH and MCOA. Data curation was carried out by CTN, PSN, JF, IH, EY, SP, MMF, EOE, GMP, CMT, JNT, AM, EZ, JFNT, LN, VF, CNN, YT, DNB, BAM and HH. Formal data analysis was performed by CTN, PSN, JF, RGE, IH, AA-MBY, EY, SP, MMF, EOE, GMP, CMT, JNT, AM, EZ, JFNT, VF, CNN and YT. The investigation phase involved CTN, PSN, JF, IH, NIM, SP and MCOA. Methodological design was performed by CTN, PSN, JF, IH, AA-MBY, NIM and MCOA. Software support was provided by CTN, PSN, JF, IH, EY, GMP, CMT and JNT. Validation of results was conducted by CTN, PSN, JF, IH, AA-MBY, NIM, EY, SP, MMF, EOE, GMP, CMT, JNT, AM, JFNT, VF, CNN, YT and BAM. The original draft of the manuscript was written by CTN, PSN, JF and IH, while all authors contributed to reviewing and editing the final version of the manuscript. CTN is responsible for the overall content as the guarantor.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.



**Patient consent for publication** Consent obtained directly from patient(s).

**Ethics approval** Ethical approval for this study was granted by the National Committee for Ethics in Human Health Research (Reference: 2023/02/1526/CE/CNERSH/SP). All participants were provided with detailed information regarding the study's objectives, potential risks and benefits through an information note. Written informed consent form was obtained from each participant prior to enrolment (online supplemental file 2).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iD

Christian Taheu Ngounouh <http://orcid.org/0000-0002-4781-0225>

#### REFERENCES

- 1 Peeling RW, Mabey D, Kamb ML, *et al*. Syphilis. *Nat Rev Dis Primers* 2017;3:17073.
- 2 Soreng K, Levy R, Fakile Y. Serologic Testing for Syphilis: Benefits and Challenges of a Reverse Algorithm. *Clin Microbiol News* 2014;36:195–202.
- 3 Newman L, Rowley J, Vander Hoorn S, *et al*. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS One* 2015;10:e0143304.
- 4 CDC. STI Statistics, 2024. Available: <https://www.cdc.gov/sti-statistics/annual/summary.html>
- 5 Trope LA, Wijesooriya NS, Broutet N, *et al*. Reaching beyond pregnant women to eliminate mother-to-child transmission of syphilis in Africa. *Expert Rev Anti Infect Ther* 2014;12:705–14.
- 6 Hook EW. Syphilis. *Lancet* 2017;389:1550–7.
- 7 Braccio S, Sharland M, Ladhani SN. Prevention and treatment of mother-to-child transmission of syphilis. *Curr Opin Infect Dis* 2016;29:268–74.
- 8 Eickhoff CA, Decker CF. Syphilis. *Dis Mon* 2016;62:280–6.
- 9 Geusau A, Kittler H, Hein U, *et al*. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory reactions in an analysis of 300,000 sera. *Int J STD AIDS* 2005;16:722–6.
- 10 Benzaken AS, Galbán García E, Sardinha JCG, *et al*. Rapid tests for diagnosing syphilis: validation in an STD clinic in the Amazon Region, Brazil. *Cad Saude Publica* 2007;23 Suppl 3:S456–64.
- 11 Henao-Martínez AF, Johnson SC. Diagnostic tests for syphilis. *Neur Clin Pract* 2014;4:114–22.
- 12 Solaimalai D, Rathore S, Beck MM, *et al*. Enzyme-linked immunosorbent assay (ELISA) versus Venereal Disease Research Laboratory test (VDRL) and rapid plasma reagin test (RPR) for screening of syphilis in pregnant women. *Int J Gynecol Obstet* 2020;150:103–7.
- 13 García Luna JA, Romero-Rosas N, Silva Peña SA, *et al*. Diagnostic performance of two rapid tests for syphilis screening in people living with HIV in Cali, Colombia. *PLoS ONE* 2023;18:e0282492.
- 14 Gallo Vaulet L, Morando N, Casco R, *et al*. Evaluation of the utility of a rapid test for syphilis at a sexually transmitted disease clinic in Buenos Aires, Argentina. *Sci Rep* 2018;8:1–6.
- 15 Kamolrattana R, Songtaweasin WN, Suchartlikitwong P, *et al*. Good performance of syphilis rapid diagnostic test kits among young key populations in Thailand. *Int J STD AIDS* 2023;34:702–9.
- 16 PDFCOFFEE. Leslie Kish-Survey Sampling-John Wiley & Sons, Inc, 1965. Available: <https://pdfcoffee.com/leslie-kish-survey-sampling-john-wiley-amp-sons-inc-1965-5-pdf-free.html>
- 17 Home - Biorex Diagnostics - Primary Diagnostics Innovation, Available: <https://biorexdiagnostics.com/>
- 18 Schoonjans F. MedCalc's Diagnostic test evaluation calculator, Available: [https://www.medcalc.org/calc/diagnostic\\_test.php](https://www.medcalc.org/calc/diagnostic_test.php)
- 19 Lodiogo DK, K Bior B, W Dumo G, *et al*. Field evaluation of SD BIOLINE HIV/Syphilis Duo assay among pregnant women attending routine antenatal care in Juba, South Sudan. *PLoS One* 2018;13:e0205383.
- 20 Van Den Heuvel A, Smet H, Prat I, *et al*. Laboratory evaluation of four HIV/syphilis rapid diagnostic tests. *BMC Infect Dis* 2019;19:1.
- 21 Zhang Y, Goh SM, Mello MB, *et al*. Improved rapid diagnostic tests to detect syphilis and yaws: a systematic review and meta-analysis. *Sex Transm Infect* 2022;98:608–16.
- 22 Shimelis T, Tadesse E. The diagnostic performance evaluation of the SD BIOLINE HIV/syphilis Duo rapid test in southern Ethiopia: a cross-sectional study. *BMJ Open* 2015;5:e007371.
- 23 Arai C, Lemos-Machado JA, Aun MV, *et al*. Sensitivity and specificity of a syphilis rapid diagnostic test in blood donors' samples. *Braz J Infect Dis* 2023;27:103689.