

RESEARCH

Open Access



# Diagnostic performance evaluation of the Abbott Bioline™ HIV/Syphilis Duo rapid test at the national public health laboratory, Yaounde-Cameroon

Philippe Salomon Nguwoh<sup>1,2,3,4,5,6</sup>, Christian Taheu Ngounouh<sup>1,2,4,5,6\*</sup>, René Ghislain Essomba<sup>1,7,8</sup>, Ibrahima Halilou<sup>1</sup>, Amina Al-Mayé Bit Younouss<sup>1</sup>, Nafissatou Ibnou Moussa<sup>1</sup>, Emile Yuosembom<sup>1</sup>, Solange Pefouakeu Pepa<sup>1</sup>, Marcel Mbarga Foe<sup>1</sup>, Emmanuel Orock Eta<sup>1</sup>, Cressence Fouda<sup>1</sup>, Guianni Mpiwouo Panyere<sup>1</sup>, Jérôme Ngantchui Tchuisseu<sup>1</sup>, Giraud Donfack Ngueguim<sup>1</sup>, Aboubakar Moussa<sup>9</sup>, John Francois Ndombol Tembten<sup>10</sup>, Lydie Nyatte<sup>11</sup>, Victor Fondoh<sup>12,13</sup>, Constance Nyanda Nyeng<sup>14</sup>, Youssoufa Taoufick<sup>15</sup>, Dodo Nyako Balkissou<sup>15</sup>, Blaise Akenji Mboringong<sup>1</sup>, Hamsatou Hadja<sup>1</sup>, Marie Claire Assoumou Okomo<sup>1,7</sup>, Désiré Tchoffo<sup>2,3,6</sup> and Henri Lucien Kamga<sup>16</sup>

## Abstract

**Background** The Bioline™ HIV/Syphilis Duo rapid diagnostic test (RDT) is the first World Health Organization (WHO) prequalified Duo RDT for the simultaneous detection of human immunodeficiency virus (HIV) and *Treponema pallidum* antibodies in human blood samples. Several studies have shown the satisfactory diagnostic performance of this test among vulnerable populations such as pregnant women. However, the diagnostic performance of this Duo test is scarce in the general population in Cameroon. The present study aimed to assess the diagnostic performance of the Abbot Bioline™ HIV/Syphilis Duo RDT in Cameroon.

**Methods** A laboratory based cross-sectional and comparative study was conducted from January, 6th to June, 19th 2024 on serum/plasma collected from adult individuals (aged:  $\geq 21$  years), recruited consecutively in health facilities in eight regions of Cameroon. The blood samples were received and stored at the National Public Health Laboratory (NPHL). The Bioline™ HIV 1/2 test was performed following the national testing algorithm with Determine™ HIV 1/2 (first test) and KHB Shanghai HIV1/2 (second test). Meanwhile, the performance of the Bioline™ syphilis test was compared with the Hightop RDT and *Treponema pallidum* hemagglutination assay (TPHA). The enzyme linked immunosorbent assay (ELISA) was used as the reference assay. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Cohen's kappa ( $\kappa$ ) value were calculated for each marker against the reference assay within a 95% confidence interval (CI) using Epi-info version 7, MedCalc and QuickCalcs for online statistical analysis.

\*Correspondence:  
Christian Taheu Ngounouh  
taheuchristian@gmail.com

Full list of author information is available at the end of the article



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

**Results** A total of 534 blood samples were tested at the NPHL with 70.97% (379/534) from females and 29.03% (155/534) from males. For HIV diagnosis, the Bioline™ HIV1/2 test had a sensitivity of 99.70% (95% CI: 98.30–99.9), specificity of 98.5% (95% CI: 95.58–99.5), PPV of 99.1% (95% CI: 97.40–99.7), NPV of 99.5% (95% CI: 97.3–99.9), accuracy of 99.1% (95% CI: 97–99.7) and Cohen's kappa of 98.4% (95% CI: 96.7–99.9). For syphilis diagnosis, the Bioline™ test had a sensitivity of 95% (95% CI: 73.7–99.7), specificity of 100% (95% CI: 99.1–100), PPV of 100% (95% CI: 79.1–100), NPV of 99.8% (95% CI: 98.7–100), accuracy of 99.9% (95% CI: 99–100), and Cohen's kappa of 97.3% (95% CI: 92.1–100).

**Conclusion** The results of this study show that the Abbot Bioline™ HIV/Syphilis Duo RDT has a good performance. Therefore, we suggest that this test could be suitable for use in the diagnosis of HIV and syphilis.

**Keywords** Bioline™ HIV/Syphilis, RDT, Sensitivity, Specificity, PPV, NPV, Accuracy

## Background

HIV and syphilis are both sexually transmitted infections (STIs) that persists as a significant public health problem despite the accessibility of an effective and affordable treatment for more than ten years [1–3]. These two STIs share similar transmission routes including bodily fluids of an infected person, blood transfusions, breast milk, semen and vaginal secretions, from mother to child [3–6]. The incidence of syphilis has been increasing in several settings, singularly in countries with limited resources and among vulnerable populations, including pregnant women and people living with HIV [7–9]. WHO has recently called for the triple elimination of mother-to-child transmission (MTCT) of HIV, syphilis and hepatitis B virus, with new strategies and integrated monitoring and evaluation activities [5]. Thus, screening for HIV and syphilis in pregnant women is recommended by the WHO to prevent MTCT, to reduce the morbidity and mortality associated with these undiagnosed and untreated infections [10].

In Cameroon, HIV and syphilis remain major public health concerns for decades. The burden of both infections varies widely across regions and population groups [11–15]. While HIV prevalence has significantly decreased in the general population from 5.5% in 2004 to 2.7% in 2018, it remains relatively stable among pregnant women, as reported in the 2017–2018 Cameroon population-based HIV impact assessment (CAMPHIA) survey [16]. Meanwhile, syphilis prevalence remains high and shows an important upward trend among pregnant women, increasing from 0.6% in 2009 to 5.7% in 2017 [17]. To strengthen early detection and improve prevention of MTCT, the WHO recommends the use of HIV/syphilis dual RDTs as the initial screening tool among blood donors and pregnant women.

The Bioline™ HIV/Syphilis Duo rapid test, developed by Standard Diagnostics (now Abbott), received WHO prequalification in November 2015, becoming the first dual HIV–syphilis point-of-care assay approved for global use [18]. Since then, it has been widely implemented in antenatal care (ANC) and prevention of mother-to-child transmission (PMTCT) programs,

particularly in low-resource settings. In Cameroon, the Duo test has been progressively introduced through pilot initiatives and ANC programs led by the Ministry of Public Health and implementing partners. Findings from ministerial field evaluations conducted in Yaounde and Douala indicated good feasibility but reported variations in sensitivity under real-world conditions [19].

Several laboratory and field studies across Africa and globally have demonstrated high diagnostic accuracy of the Duo test, especially for HIV (sensitivity and specificity  $\geq 99\%$ ) and strong performance for syphilis detection [10, 20]. A meta-analysis by Gliddon et al. (2017), including 18 evaluation studies, ranked the Bioline™ Duo test among the best-performing dual HIV/syphilis RDTs worldwide. The assay was also reported to be highly acceptable for point-of-care use due to its short turnaround time and reduced cost [21]. Further investigations by Lodiongo et al. (2018) reported HIV sensitivity and specificity of 100% and 99.5%, and syphilis sensitivity and specificity of 97.6% and 96%, respectively [22]. Similarly, a study in Ethiopia found both HIV sensitivity and specificity to be 100% and 99.5% [23]. These findings support the Bioline™ HIV/Syphilis Duo test as a validated, robust dual rapid diagnostic tool suitable for resource-limited and point-of-care settings. However, despite its progressive use, data on its diagnostic performance among pregnant women and the general population in Cameroon remain limited.

## Study objectives

The objectives of this study were: (i) to compare the diagnostic performance of the Bioline™ HIV/Syphilis Duo RDT with Determine HIV 1/2 and the ELISA HIV 1/2 used as a reference assay, (ii) to compare the diagnostic performance of the Bioline™ HIV/Syphilis Duo RDT with Hightop RDT, TPHA and the ELISA syphilis used as a reference assay. (iii) To compare the receiver operating characteristic (ROC) curve of the Bioline™ HIV1/2 RDT and Bioline Syphilis RDT.

## Materials and methods

### Study design and setting

From January, 6th to June, 19th 2024 a laboratory based cross-sectional and comparative study was conducted to assess the diagnostic performance of the Bioline™ HIV/Syphilis Duo RDT with blood samples obtained from adult individuals (aged:  $\geq 21$  years) health facilities in the eight zones geographical areas of Cameroon. The samples collected in health facilities were sent to the NPHL. The laboratory is located behind the training schools of the Ministry of Public Health, precisely in the Messa neighborhood, rue Rudolph Douala Manga Bell, Cité-verte health district, Mfoundi department, Yaounde two sub-division, centre region of Cameroon.

### Study population and data sources

The study included individuals of both sexes (male and female) who were at least 21 years of age and had either positive or negative HIV/Syphilis status. All participants provided informed consent to participate in the study. Exclusion criteria were (i) all individual with a non-compliant biological sample (insufficient sample or hemolysis), (ii) incomplete information questionnaire, and (iii) individual who chose to withdraw from the study at any time. Data were obtained from individuals aged 21 to 81 years, who were consecutively recruited from screening sites, treatment units and blood banks. Participants characteristics were collected through a face-to-face interview using structured questionnaire (supplement information S1) that included both quantitative (e.g. age) and qualitative (e.g. sex, HIV status...) variables. Data collected were recorded in an MS Excel spreadsheet and subsequently analysed using Epi-info v. 7 software.

### Sampling technique and sample size determination

The sampling technique used in this study was non probabilistic and the minimal sample size was determined using Kish Leslie formula:  $N = Z_{\alpha}^2 p (1-p) / d^2$  [24]. Using  $Z$  = the standard deviation of 1.96 (95% confidence interval) and  $d$  = allowable error (5%). For the Sensitivity (Se),  $N_{\text{positive}} = Z_{\alpha}^2 \times Se (1-Se) / d^2$  and the Specificity (Sp),  $N_{\text{negative}} = Z_{\alpha}^2 \times Sp (1-Sp) / d^2$  with Estimated Se = 99% and Sp = 98%. Then,  $N_{\text{positive}} = 15.21$  and  $N_{\text{negative}} = 15.06$ .

### Sample collection sites

Blood samples obtained from eight regions of the country: Littoral, West, South, South-West, North, Far-North, North-West, and East. Samples were collected from individuals at the HIV diagnostic sites, blood banks, and care units. The sites from which the samples were collected included: Nkongsamba regional hospital for the Littoral Region, Foumban district hospital for the West Region, Ebolowa regional hospital for the South Region, Limbe regional hospital for the South-West Region, Garoua

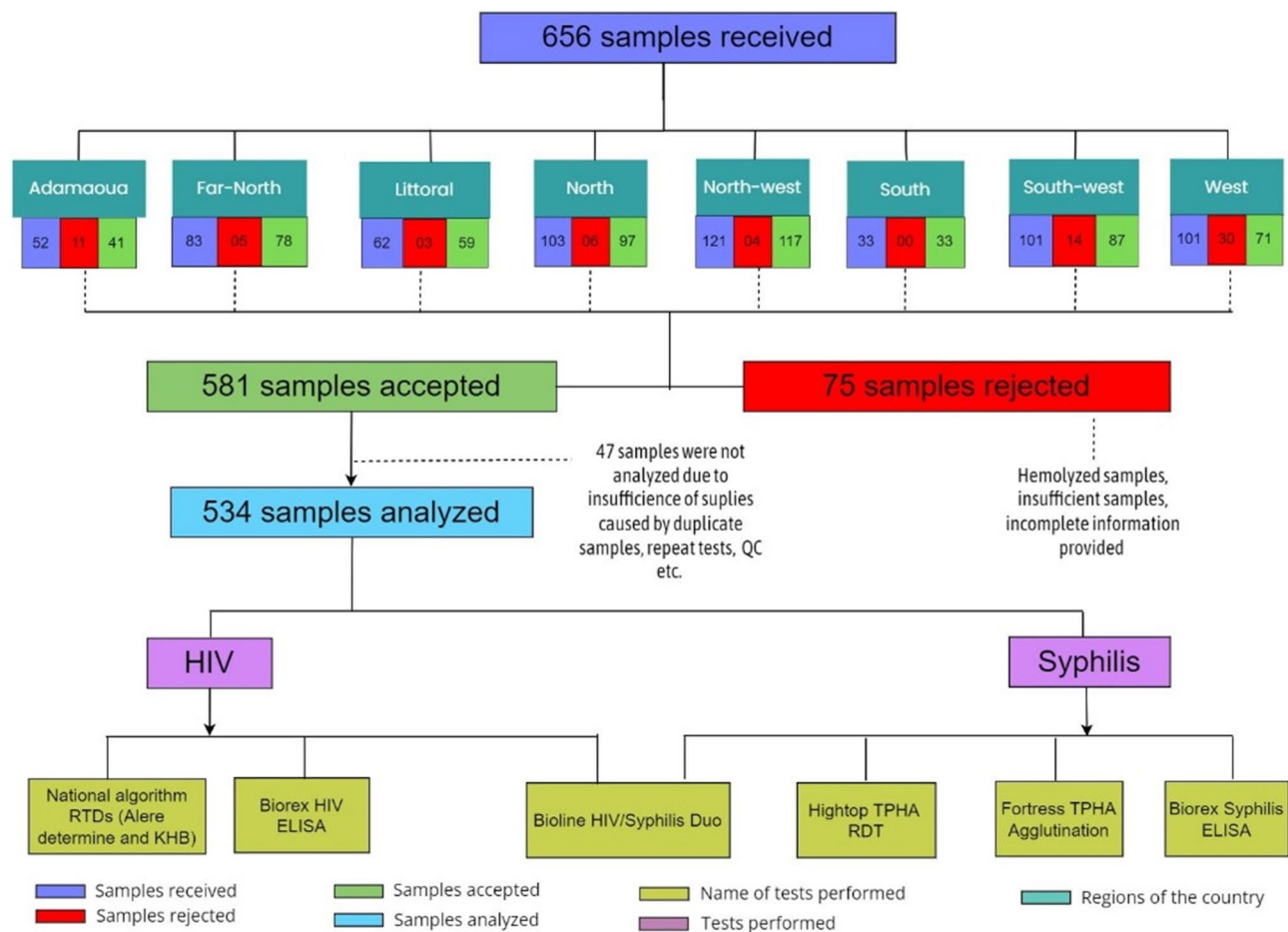
regional hospital for the North Region, Maroua regional hospital for the Far-North, Bamenda regional hospital for the North-West and Bertoua regional hospital for the East Region (Fig. 1).

### Collection, packaging and transport of blood samples

A total of four milliliters (4 ml) of whole blood was drawn from each participant by venipuncture using ethylene diamine tetra acetate (EDTA) and dried vacutainer tubes. The blood samples collected in both tubes were identified by red and blue colours respectively. Each sample was coded by a unique identifier number, which included the abbreviation of the region from which it was collected. The abbreviation used were: WT, SO, NO, FN, LT, NW, SW, AD respectively for the West, South, North, Far-North, Littoral, North-West, South-West, and Adamawa regions. Blood samples were centrifuged, to separate plasma and serum which was then collected in 2 ml cryovials. Aliquots were stored at  $-20$  °C before being transported to trucking agencies. Each blood sample was packaged using the triple packaging system according to WHO recommendations with ice packs frozen in coolers to maintain the cold chain until the place of manipulation. All packaged samples were transported to the NPHL by laboratory personnel within minutes of packaging by road transport. The cold chain was maintained during transport to the NPHL. Each sample was accompanied by the information of the study participants, including age, sex, HIV status.... Upon arrival at the NPHL, samples were stored in the freezer at  $-20$  °C until analysis.

### Sample analysis procedure at the NPHL

In the laboratory, all specimens collected from study participants were initially tested for HIV using the Cameroonian national testing algorithm. This algorithm involves sequential testing with two RDTs, followed by an ELISA assay for either confirmatory or additional testing (Fig. 2A). Each sample was initially tested using the Determine HIV 1/2 assay (Test 1 [T1] from the Abbott Laboratory, lot no. 0000736321, manufactured on 07/06/2023 and expiry date 14/11/2024), followed by the KHB Shanghai HIV 1/2 assay (Test 2 [T2] from Shanghai Kehua Bio-engineering Co. Ltd., lot no. W401230511, manufactured on 06/05/2023 and expiry date 25/05/2025) if T1 was positive. All specimens positive for the Determine HIV1/2 assay were also positive for the KHB assay (i.e. no discordant results were observed). All samples with negative or discordant RDT results, as well as all positive samples, underwent further testing using the Biorex ELISA 1/2 (Biorex Diagnostics Ltd, Lot: 2302-4, expiry date: 07/2024) [25]. This test was performed if T1 and T2 were positive or negative. Each specimen was also tested independently of the national algorithm using the Bioline™



**Fig. 1** A chart flow diagram illustrating the samples collection sites

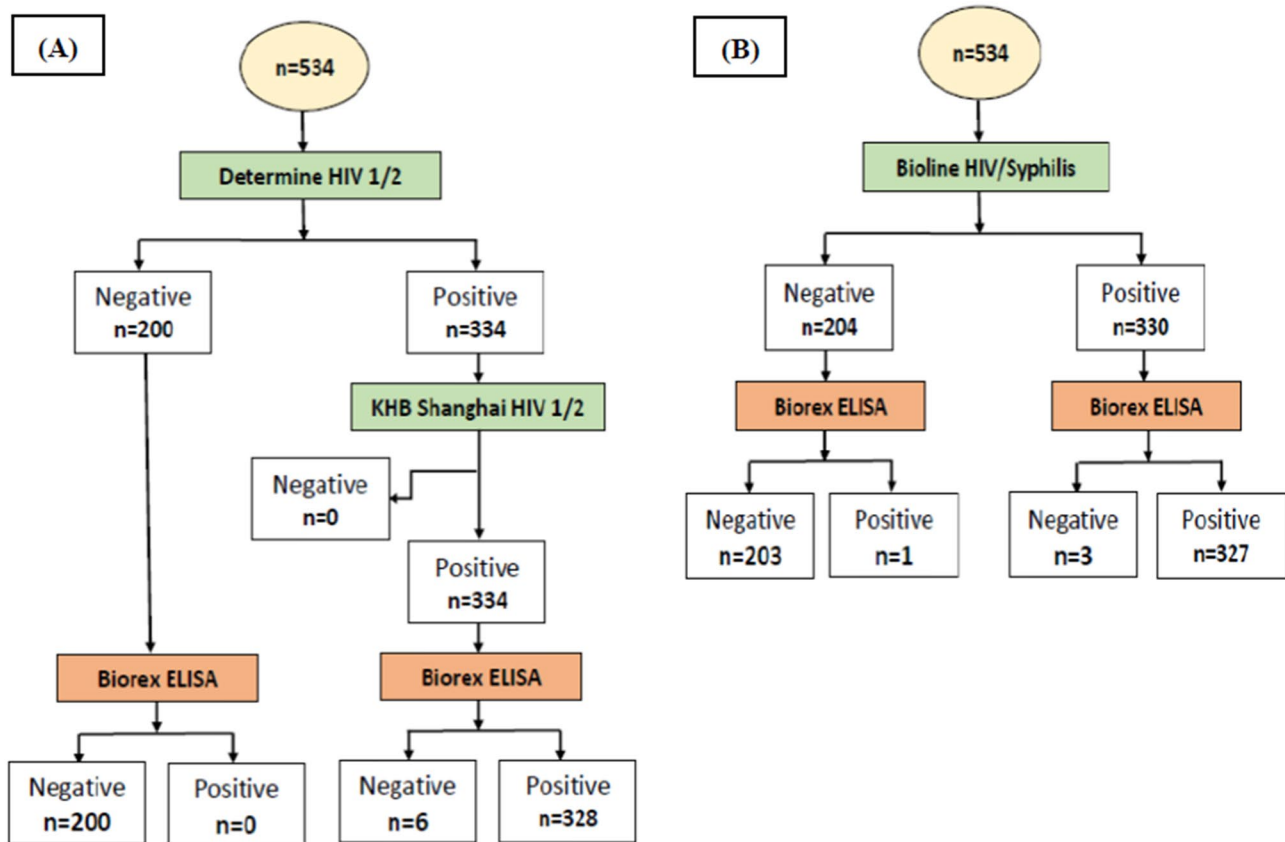
HIV/Syphilis Duo RDT (Abbott Rapid Dx International Limited; Lot No. 06ADH978REF06FK35; manufacture date:22/12/2022; expiry date: 20/12/2024). All samples were then confirmed using the Biorex HIV 1/2 (Fig. 2B).

For syphilis, each serum/plasma was tested using Hightop RDT (from Qingdao Hightop Biotech Co, Ltd, REF: H133, Lot: TP1220904, manufacture date: 2022/09/15 and expiration date:2024/09/14), TPHA (from Fortress Diagnostics Ltd, Lot: TH-2303-4, manufacture date: 2023/3 and expiration date: 2024/10), Bioline syphilis (from Abbott Rapid Dx International Limited, Lot: 06ADH978REF06FK35, manufacture date: 20221222 and expiration date: 20241220), and Biorex syphilis (from Biorex Diagnostics Ltd, Lot: TP20210-1, manufacture date: 2022/10 and expiration date: 2024/06) [25] according to the manufactured instructions (Fig. 3).

**Statistical analysis**

Data collected from health facilities across the eight regions of the country were received at the NPHL. The data collected was verified and entered in a Microsoft Excel (MS) version 97-2003 sheet. The data was then

exported to the Epi-info version 7 statistical analysis software. To assess diagnostic performances, 2 × 2 contingency tables were created comparing the results of each RDT to those of the reference assay (ELISA). Categorical variables were analysed as proportions, calculated by dividing the frequency of each response by the total sample size, and expressed as percentages. Diagnostic performance including sensitivity, specificity, PPV, NPV, accuracy, and Cohen’s kappa coefficient were calculated using validated online statistical tools such as MedCalc and QuickCalcs [26, 27]. All results were presented with 95% confidence intervals (95% CI) to reflect the precision of the estimates. Additionally, the ROC curve was plotted for each test to evaluate its ability to distinguish between positive and negative cases using MedCalc [27]. The Area Under the Curve (AUC) was computed as a quantitative measure of this discriminative capacity. An AUC value close to 1.0 was interpreted as indicating excellent diagnostic performance.



**Fig. 2** A chart flow diagram illustrating the laboratory procedure HIV tests with 534 blood samples tested [Determine vs. KHB (A), Bioline™ HIV/Syphilis Duo (B)]

**Results**

**Characteristics of the study population**

A total of 534 blood samples were analysed at the NPHL, collected from individuals across eight regions of the country. Among the participants, 70.97% (379/534) were females and 29.03% (155/534) were males. Participant ages ranged from 21 to 81 years, with a median age of 37 years and an interquartile range (IQR) of 28 to 47 years.

**Assay results of the Bioline™ HIV/Syphilis duo and determine HIV 1/2 RDT compared to the ELISA HIV 1/2**

A total of 534 samples were tested using the Determine™ HIV1/2 RDT. All 328 HIV-positive samples were correctly identified by the Determine™ HIV1/2 RDT, yielding a true positive (TP) rate of 100% (328/328) and no false negatives (FN, 0/328). Among the 206 HIV-negative samples, 97.08% (200/206) were correctly classified as true negatives (TN), while 2.91% (6/206) were identified as false positives (FP). For the Bioline™ HIV1/2 RDT, 327 out of 328 HIV-positive samples were correctly identified, resulting in a TP rate of 99.70%. One sample (0.30%) was misclassified as a FN. Among the 206 HIV-negative samples, 203 (98.54%) were correctly identified as TN,

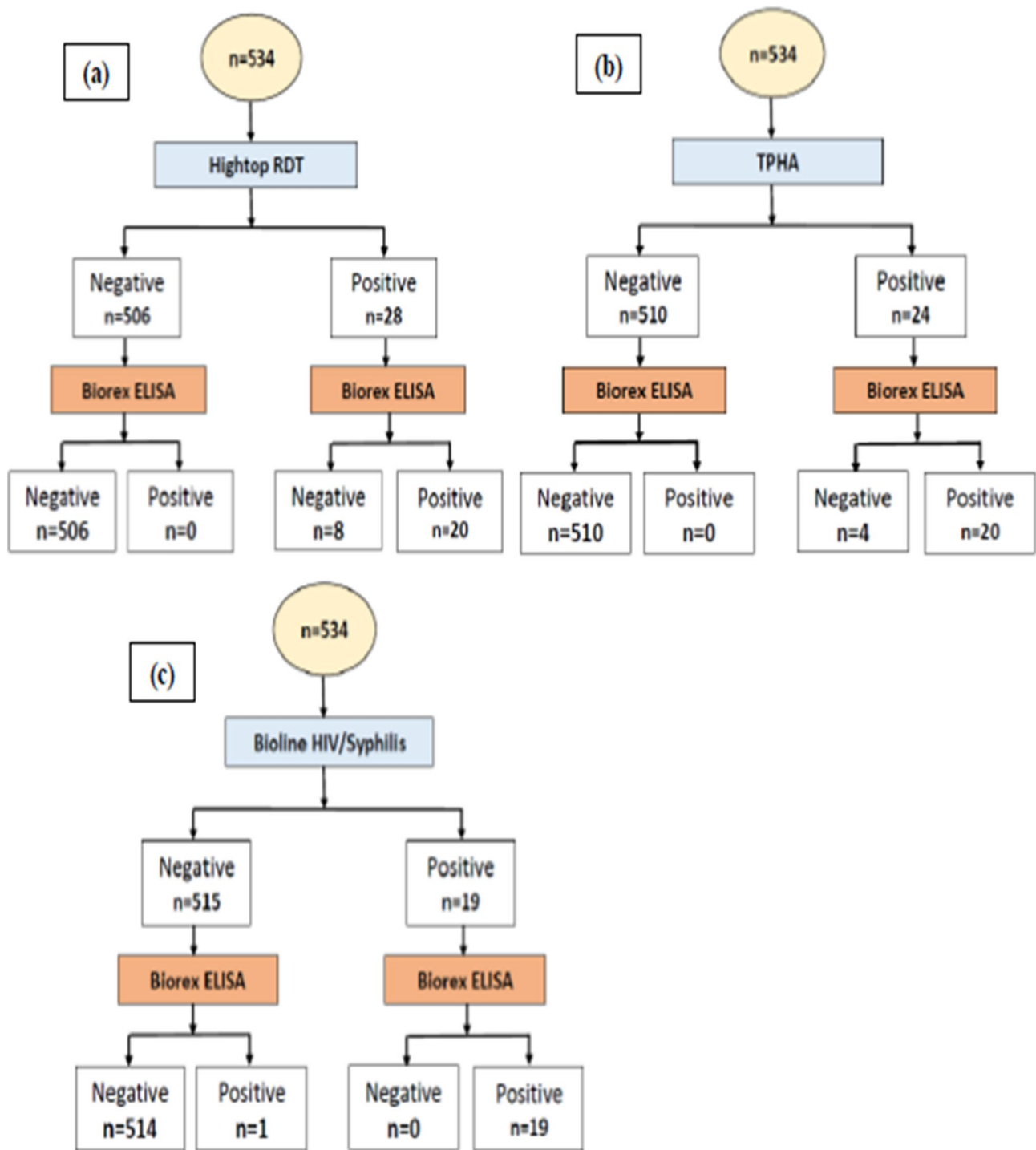
while 3 samples (1.46%) were incorrectly classified as FP (Table 1).

**Diagnostic performance of the Bioline™ HIV/Syphilis duo RDT and determine HIV 1/2**

The diagnostic performance of the Determine™ HIV1/2 RDT revealed a sensitivity of 100% (95% CI: 98.8–100) and a specificity of 97.10% (95% CI: 93.8–98.7). The PPV was 98.2% (95% CI: 96.1–99.2), and the NPV was 100% (95% CI: 98.1–100). The test demonstrated an overall accuracy of 99% (95% CI: 97–100), with a Cohen’s kappa coefficient of 98.4% (95% CI: 96.7–99.9). For the Bioline™ HIV1/2 RDT, the sensitivity was 99.70% (95% CI: 98.3–99.9), and the specificity was 98.5% (95% CI: 95.58–99.5). The PPV was 99.1% (95% CI: 97.4–99.7), and the NPV was 99.5% (95% CI: 97.3–99.9). The accuracy of the test was 99.1% (95% CI: 97–99.7), and the kappa coefficient was 98.4% (95% CI: 96.7–99.9) (Table 2).

**Assay results of the Bioline™ HIV/Syphilis duo RDT with hightop RDT and TPFA compared to the ELISA syphilis**

Out of 534 samples tested, the Hightop RDT identified all 20 TP cases (100%), with no FN, 8 FP (1.56%), and 506 TN (98.44%). The TPFA test also detected all 20 TP



**Fig. 3** A chart flow diagram illustrating the laboratory procedure syphilis tests with 534 blood samples tested [Hightop RDT (a), TPHA (b), Bioline HIV/Syphilis (c)]

cases (100%) with no FN (0%), 4 FP (0.78%), and 510 TN (99.22%). For the Bioline™ Syphilis RDT, 19 out of 20 TP cases were correctly identified (95%), with 1 FN (5%), no FP (0%), and all 514 negative samples correctly classified as TN (100%) (Table 3).

**Diagnostic performance of the Bioline™ HIV/Syphilis duo RDT with hightop and TPHA**

The Hightop RDT demonstrated a diagnostic sensitivity of 100% (95% CI: 83.9–100), and a specificity of 98.4% (95% CI: 97.0–99.2), The PPV was 71.4% (95% CI: 52.9–84.7), while the NPV reached 100% (95% CI: 99.2–100),

**Table 1** Assay results of the Bioline™ HIV/Syphilis duo and determine HIV 1/2 RDT compared to the ELISA HIV 1/2

RDTs	Reference assay			Total
	Results	Positive	Negative	
Determine HIV	Positive	328 (TP)	6 (FP)	334
	Negative	0 (FN)	200 (TN)	200
	Total	328	206	534
Bioline HIV	Positive	327 (TP)	3 (FP)	330
	Negative	01 (FN)	203 (TN)	204
	Total	328	206	534

Legend: TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative

The overall diagnostic accuracy was 98.5% (95% CI: 97–100), with a Cohen’s kappa coefficient of 82% (95% CI: 70.77–94.37). TPHA shows a sensitivity of 100% (95% CI: 83.9–100) and a specificity of 99.2% (95% CI: 98.0–99.7). The PPV and NPV were 83.3% (95% CI: 64.0–93.3) and 100% (95% CI: 99.3–100), respectively. Diagnostic accuracy was estimated at 99.4% (95% CI: 98–100), with a kappa coefficient of 90.5% (81.3–99.7). The Bioline syphilis exhibited a sensitivity of 95% (95% CI: 76.4–99.1) and a specificity of 100% (95% CI: 99.3–100). The PPV was 100% (95% CI: 83.2–100), and the NPV was 99.8% (95% CI: 98.9–100). The overall accuracy was 99.9% (95% CI: 99–100) and the kappa coefficient was 0.9734 (95% CI: 0.921–1.00) (Table 4).

**Comparison between the ROC curve of the Bioline™ HIV1/2 RDT and Bioline Syphilis RDT**

The ROC curve for the Bioline™ HIV1/2 RDT (Fig. 4A), with a sensitivity of 99.7% and specificity of 98.5%, lies near the upper-left corner, indicating excellent diagnostic accuracy. The estimated AUC of 0.995 reflects a near-perfect ability to distinguish between HIV-positive and HIV-negative individuals, confirming the test’s strong

**Table 3** Assay results of the Bioline™ HIV/Syphilis duo RDT with hightop RDT and TPHA compared to the ELISA syphilis

Tests	Reference assay			Total
	Results	Positive	Negative	
Hightop	Positive	20 (TP)	8 (FP)	28
	Negative	0 (FN)	506 (TN)	506
	Total	20	514	534
TPHA	Positive	20 (TP)	4 (FP)	24
	Negative	0 (FN)	510 (TN)	510
	Total	20	514	534
Bioline syphilis	Positive	19 (TP)	0 (FP)	19
	Negative	1 (FN)	514 (TN)	515
	Total	20	514	534

Legend: TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative

overall performance. With a sensitivity of 95.0% and specificity of 100%, the ROC curve for the Bioline™ Syphilis RDT (Fig. 4B) lies near the top-left corner, reflecting excellent diagnostic performance. The estimated AUC of 0.975 indicates strong discriminatory power, meaning the test can accurately distinguish between syphilis-positive and negative individuals in 97.5% of cases (Fig. 4).

**Discussion**

This cross-sectional comparative study aimed to evaluate the diagnostic performance of the Bioline™ HIV/Syphilis Duo RDT, using 534 serum and plasma samples. For the HIV detection, the Bioline™ test demonstrated a sensitivity of 99.7% (95% CI: 98.3–99.9) and a specificity of 98.5% (95% CI: 95.6–99.5). By comparison, the Determine™ HIV 1/2 test demonstrated 100% sensitivity (95% CI: 98.8–100) and 97.1% specificity (95% CI: 93.8–98.7). Therefore, the Determine™ HIV 1/2 test was slightly more sensitive, while the Bioline™ HIV/Syphilis Duo test showed better specificity. These results are consistent with those of

**Table 2** Diagnostic performance of the Bioline™ HIV/Syphilis duo RDT and determine HIV1/2

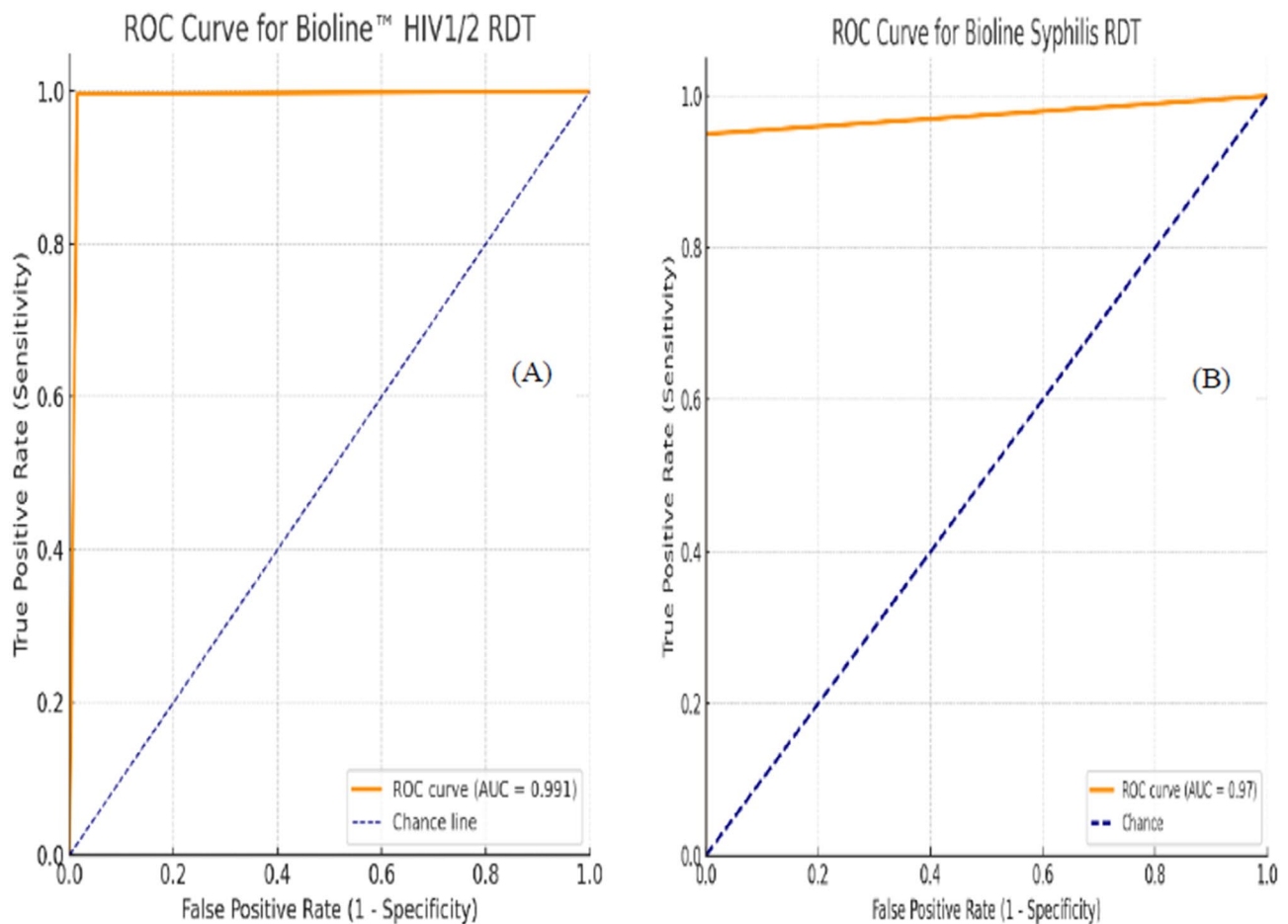
HIV1/2 RDTs	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	Accuracy 95% CI	Kappa 95% CI
Determine	100% (95% CI: 98.8–100)	97.10% (95% CI: 93.8–98.7)	98.2% (95% CI: 96.1–99.2)	100% (95% CI: 98.1–100)	99% (95% CI: 97–100)	98% (95% CI: 96.7–99.9)
Bioline HIV	99.70% (95% CI: 98.30–99.9)	98.5% (95% CI: 95.58–99.5)	99.1% (95% CI: 97.40–99.7)	99.5% (95% CI: 97.3–99.9)	99.1% (95% CI: 97.99.7)	98% (95% CI: 96.7–99.9)

Legend: PPV: positive predictive value, NPV: negative predictive value, CI: Confidence Interval

**Table 4** Diagnostic performance of the Bioline™ HIV/Syphilis duo RDT with hightop and TPHA

RDTs	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	Accuracy 95% CI	Kappa 95% CI
Hightop	100% (95% CI: 83.9–100)	98.4% (95% CI: 97.0–99.2)	71.4% (95% CI: 52.9–84.7)	100% (95% CI: 99.2–100)	98.5% (95% CI: 97–100)	82% (95% CI: 70.77–94.37)
TPHA	100% (95% CI: 83.9–100)	99.2% (95% CI: 98–99.7)	83.3% (95% CI: 64.0–93.3)	100% (95% CI: 99.3–100)	99.4% (95% CI: 98–100)	90.5% (95% CI: 81.3–99.7)
Bioline syphilis	95% (95% CI: 76.4–99.1)	100% (95% CI: 99.3–100)	100% (95% CI: 83.2–100)	99.8% (95% CI: 98.9–100)	99.9% (95% CI: 99–100)	97.34% (95% CI: 92.1–100)

Legend: PPV: positive predictive value, NPV: negative predictive value, CI: Confidence Interval



**Fig. 4** The ROC curve for the Bioline™ HIV1/2 RDT (A) and Bioline Syphilis RDT (B)

previous studies by Shakya et al. (2016) and Omoding et al. (2014), which also confirmed the excellent accuracy of the SD Bioline™ HIV/Syphilis Duo test [20, 28]. Taremwa et al., (2019) observed that the sensitivity and specificity of the Bioline™ HIV/Syphilis Duo assay were all 100.0% (95% CI: 99.5 to 100.0 and 98.6 to 100.0, respectively) [29]. Furthermore, the study conducted by Withers et al., (2019) reported that for HIV antibody testing, sensitivity and specificity were 100.0% (95% CI: 80.5–100.0%) and 100.0% (95% CI: 98.6–100.0), respectively [30]. Moreover, the sensitivity and specificity for the HIV antibody test component ( $n = 2336$ ) were estimated at 99.91% (95% CI, 99.51% and 100%) and 99.67% (95% CI, 99.16% and 99.91%), respectively according to the findings reported by Bristow et al., (2014) [10]. The study conducted by Olugbenga et al., (2018) observed a sensitivity of 100.0% (95% CI 99.7–100.0) and specificity of 99.9% (95% CI 99.7–100.0) [31]. All these studies demonstrated a good diagnostic performance of the Bioline HIV/Syphilis Duo test for the HIV diagnostic. Our findings could be explained by the population studied, as these studies have

worked with pregnant women. These results could also be justified by the sample size.

The Bioline™ HIV/Syphilis Duo test is highly accurate, with a PPV of 99.1% (95% CI: 97.4–99.7) and an NPV of 99.5% (95% CI: 97.3–99.9). The Determine™ HIV1/2 test had a PPV of 98.2% (95% CI: 96.1–99.2) and an NPV of 100% (95% CI: 98.1–100). This proves that 98.2% of the samples identified as positive were in fact positive, and that 100% of the samples identified as negative were truly negative. These tests also demonstrated notable accuracy and kappa statistics. The Determine™ HIV1/2 test had an accuracy of 99% (95% CI: 97–100) and a kappa of 98% (95% CI: 96.7–99.9), demonstrating excellent agreement with the reference assay. The Bioline™ HIV/Syphilis Duo test is highly accurate, with an accuracy of 99.1% (95% CI: 97–99.7) and a kappa of 98% (95% CI: 96.7–99.9). This further supports its reliability. Our findings corroborate with the study conducted among pregnant women attending routine antenatal care in Juba, South Sudan [22].

For the syphilis antibody testing, Bioline™ HIV/Syphilis Duo test had a sensitivity of 95% (95% CI: 76.4–99.1) and

a specificity of 100% (95% CI: 99.3–100). The test correctly identified 19 out of 20 samples positive for syphilis (19 true positives), with one false negative. All 514 samples that tested negative for syphilis were correctly identified as such, with no false positives. Similar findings were found by Shakya et al., (2016) who reported that the sensitivity of the kit for syphilis diagnosis was 95.45% (95% CI 84.86–98.74%) and specificity was 99.87% (95% CI; 99.78–99.92) [28]. Compared to several studies, the study conducted by Withers et al., (2019) reported that for syphilis diagnosis, sensitivity and specificity were 83.1% (95% CI: 71.0–91.6) and 100.0% (95% CI: 98.3–100.0), respectively [30]. Chiappe et al., (2013) demonstrated that for syphilis diagnosis, Bioline™ HIV/Syphilis had a sensitivity of 100% (198/198) and a specificity of 99.57% (465/467) [32]. Our findings could be explained by the gold standard technique used such as ELISA compared to TPHA. In this present study, the TPHA Syphilis test had a sensitivity of 100% (95% CI: 83.9–100) and a specificity of 99.2% (95% CI: 98–99.7), the PPV was 83.3% (95% CI: 64.0–93.3), the NPV was 100% (95% CI: 99.3–100), an accuracy of 99.4% (95% CI: 98–100), and a kappa of 90.5% (95% CI: 81.3–99.7). The test correctly identified all 20 syphilis-positive samples and had four false positives among the 514 negative samples. These findings are consistent with recent data indicating variability in sensitivity among syphilis tests [22] reported that while some RDTs had high specificity, their sensitivity varied significantly. The Bioline™ HIV/Syphilis Duo RDT's performance is consistent with other dual tests. Several studies evaluating the Bioline™ HIV/Syphilis Duo test in five countries reported a sensitivity of 99.3% and specificity of 99.4% for HIV, supporting the utility of dual RDTs for accurate HIV screening [10, 33].

The ROC curve analysis confirmed the excellent diagnostic performance of both the Bioline™ HIV1/2 and Bioline™ Syphilis RDTs. The Bioline™ HIV1/2 test achieved an AUC of 0.995, along with a sensitivity of 99.7% and specificity of 98.5%, indicating near-perfect ability to distinguish HIV-positive from HIV-negative individuals. Similarly, the Bioline™ Syphilis RDT showed an AUC of 0.975, with a sensitivity of 95.0% and specificity of 100%, reflecting excellent accuracy. The perfect specificity is particularly valuable in syphilis screening, helping to avoid false positives and unnecessary treatment. These findings support the integration of both tests into routine screening programs, especially in antenatal care, where early detection of HIV and syphilis can significantly reduce MTCT and improve pregnancy outcomes.

#### Study limitations

This study has several limitations. Firstly, participants were recruited solely from health facilities, which may have introduced selection bias. Those accessing

healthcare services may differ from the general population in terms of their health-seeking behaviour, awareness of their infection status, access to testing and disease stage. Consequently, the study population may be disproportionately represented by individuals who are already receiving care or have more advanced disease. This could affect the accuracy of diagnostic estimates and limit the applicability of the findings to community-based settings. Secondly, as the study was conducted across eight regions and only included adult participants, the results may be limited in their applicability to other populations, such as children and adolescents, or to adults in areas not represented. Additionally, some participants were people living with HIV who were on antiretroviral therapy and were therefore likely to have well-controlled viral loads. However, this could introduce bias, as immune status and viral suppression may affect the outcome of diagnostic tests. Therefore, the findings may not accurately reflect the performance of individuals with uncontrolled HIV or of the general population. Thirdly, despite the study including 534 samples, only 20 participants tested positive for syphilis. This small number of positive cases means that the precision of sensitivity estimates is limited, resulting in wide confidence intervals. This limitation is due to the low prevalence of HIV and syphilis in the general population, as well as the relatively short data collection period. More precise and reliable estimates of diagnostic performance could be obtained from larger studies or studies designed to include more positive cases. Fourthly, ELISA was used as the sole reference assay due to its high sensitivity and specificity, as well as its ease of standardisation. However, misclassification of some cases may have occurred due to the absence of confirmatory treponemal tests, such as FTA-ABS or polymerase chain reaction (PCR). Exclusive reliance on ELISA could lead to an overestimation of syphilis prevalence or affect the accuracy with which the Bioline™ Duo RDT measures. The inclusion of confirmatory assays is considered a best practice for diagnostic accuracy studies. The presence of both false-positive and false-negative results underlines the importance of confirmatory testing for an accurate diagnosis. Although the Bioline™ Duo RDT provides rapid results, confirmatory assays are crucial for making clinical decisions. Furthermore, routine clinical care relies on non-treponemal tests, such as the Rapid Plasma Reagin (RPR), to stage the disease, initiate treatment, and monitor the response to therapy. As this study did not incorporate these steps, the rapid test alone cannot provide information on disease activity or treatment outcomes.

## Conclusion

The Bioline™ HIV/Syphilis Duo RDT performed excellently in detecting HIV, confirming its effectiveness as a screening tool. The syphilis component also demonstrated acceptable diagnostic accuracy, albeit with slightly lower sensitivity than the TPHA test. The high levels of sensitivity, specificity, PPV and NPV observed, particularly for HIV, reinforce the Duo RDT's diagnostic value. Further ROC curve analysis demonstrates that both the HIV1/2 and syphilis components possess strong discriminatory power and excellent overall diagnostic accuracy. Although only 20 of the study participants tested positive for syphilis, these results still support the use of Duo RDTs in settings with limited resources. The ability to rapidly and simultaneously screen for both infections could greatly improve diagnostic efficiency and patient care in such settings. Nevertheless, confirmatory testing for syphilis and ongoing quality assurance are essential for optimal performance and clinical reliability.

## Abbreviations

ART	Antiretroviral treatment
AUC	Area Under the Curve
CI	Confidence interval
EDTA	Ethylene diamine tetra acetate
ELISA	Enzyme linked immunosorbent assay
HIV	Human immunodeficiency virus
IQR	Interquartile Range
MTCT	Mother-to-child transmission
NPHL	National public health laboratory
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
RDT	Rapid diagnostic test
ROC	Receiver Operating Characteristic
STI	Sexually transmitted infections
TPHA	Treponema pallidum hemagglutination assay
WHO	World health organization

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-026-12531-3>.

Supplementary Material 1

Supplementary Material 2

## Acknowledgements

We would like to sincerely thank all the regional hospital Directors for the authorizations granted so that this study could be conducted. Also, we sincerely thank all the laboratory technicians from different hospitals for data collection and transportation of biological samples. We would like to thank the laboratory technicians for the technical support. Moreover, our deep and sincere thanks to all participants who gave their consent to participate in the study.

## Author contributions

Philippe Salomon Nguwoh, Christian Taheu Ngounouh, Ibrahima Halilou, Hamsatou Hadja, Marie Claire Assoumou Okomo, and Henri Lucien Kamga contributed to the conceptualization, resource provision, and overall project administration. Data collection, investigation, methodological development, and formal analysis were performed by Philippe Salomon Nguwoh, Christian Taheu Ngounouh, Ibrahima Halilou, Emile Yuosembom, Solange Pefouakeu

Pepa, Marcel Mbarga Foe, Emmanuel Orock Eta, Cressence Fouda, Guianni Mpiwouo Panyere, Jérôme Ngantchui Tchuisseu, Giraud Donfack Ngueguim, Aboubakar Moussa, John Francois Ndombol Tembten, Lydie Nyatte, Victor Fondoh, Constance Nyanda Nyeng, and Youssoufa Taoufick. Software and data handling were carried out by Philippe Salomon Nguwoh, Christian Taheu Ngounouh, Ibrahima Halilou, Emile Yuosembom, Guianni Mpiwouo Panyere, Jérôme Ngantchui Tchuisseu, and Giraud Donfack Ngueguim. Visualization and validation of the data were done by René Ghislain Essomba, Amina Al-Mayé Bit Younouss, Nafissatou Ibnou Moussa, Dodo Nyako Balkissou, Blaise Akenji Mboringong, Hamsatou Hadja, Marie Claire Assoumou Okomo, Désiré Tchoffo, and Henri Lucien Kamga. Philippe Salomon Nguwoh and Christian Taheu Ngounouh drafted the initial version of the manuscript. René Ghislain Essomba, Amina Al-Mayé Bit Younouss, Nafissatou Ibnou Moussa, Dodo Nyako Balkissou, Blaise Akenji Mboringong, Hamsatou Hadja, Marie Claire Assoumou Okomo, Désiré Tchoffo, and Henri Lucien Kamga critically reviewed and revised the manuscript. All authors reviewed and approved the final manuscript.

## Funding

No funding was received for the article processing charge (APC).

## Data availability

The datasets used during the current study are available (supplement information S2).

## Declarations

### Ethics approval and consent to participate

This study obtained an ethics clearance from the National Human Health Research Ethics Committee of Cameroon (CE N°2024/05/1665/CE/CNERSH/SP from May, 7th 2024). Written informed consent was obtained from all participants before enrolment. In accordance with the Declaration of Helsinki, all participants enrolled in this study were duly informed of the minimal risks associated with blood sample collection and the potential benefits of participation through a detailed information note. For participants living with HIV and other vulnerable populations, the confidentiality was respected by assigning a unique identifying code to each individual questionnaire. Additionally, participation in the study was free without constraint.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

<sup>1</sup>National Public Health Laboratory, Ministry of Public Health, Yaounde, Cameroon

<sup>2</sup>Faculty of Health Sciences, University of Lisala, Lisala, Mongala, Democratic Republic of Congo

<sup>3</sup>Private Franco-Arab African University, Bamako, Mali

<sup>4</sup>Higher Institute of Sciences and Techniques Applied to Health, Yaounde, Cameroon

<sup>5</sup>Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon

<sup>6</sup>Centre Interuniversitaire de Recherche Pluridisciplinaire (CIREP), Kinshasa, Democratic Republic of Congo

<sup>7</sup>Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon

<sup>8</sup>School of Health Sciences, Catholic University of Central Africa, Yaounde, Cameroon

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

<sup>10</sup>Nkongsamba Regional Hospital, Ministry of Public Health, Nkongsamba, Cameroon

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

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

<sup>13</sup>Department of Public Health, Faculty of Health Sciences, University of Bamenda, Bamenda, Cameroon

<sup>14</sup>Limbe Regional Hospital, Ministry of Public Health, Limbe, Cameroon

<sup>15</sup>Garoua Regional Hospital, Ministry of Public Health, Garoua, Cameroon

<sup>16</sup>University of Dschang, Dschang, Cameroon

Received: 14 July 2025 / Accepted: 5 January 2026

## References

- Marcus U, Ort J, Grenz M, Eckstein K, Wirtz K, Wille A. Risk factors for HIV and STI diagnosis in a community-based HIV/STI testing and counselling site for men having sex with men (MSM) in a large German City in 2011–2012. *BMC Infect Dis*. 2015;15(1):14.
- Peeling RW, Mabey D, Kamb ML, Chen XS, Radolf JD, Benzaken AS, Syphilis. *Nat Rev Dis Primers*. 2017;3(1):1–21.
- Peeling RW, Mabey D, Chen XS, Garcia PJ. Syphilis Lancet. 2023;402(10398):336–46.
- Tudor ME, Al Aboud AM, Leslie SW, Gossman W. In: StatPearls T, editor. Syphilis. Island (FL): StatPearls Publishing; 2024.
- Bell L, van Gemert C, Allard N, Brink A, Chan PL, Cowie B, et al. Progress towards triple elimination of mother-to-child transmission of HIV, hepatitis B and syphilis in Pacific Island countries and territories: a systematic review. *Lancet Reg Health West Pac*. 2023;35:100740.
- Arrieta AC, Singh J. Congenital syphilis. *N Engl J Med*. 2019;381(22):2157.
- Kojima N, Klausner JD. An update on the global epidemiology of syphilis. *Curr Epidemiol Rep*. 2018;5(1):24–38.
- Nanoudis S, Pilalas D, Tziouvanaki T, Constanti M, Markakis K, Pagioulas K, et al. Prevalence and treatment outcomes of syphilis among people with human immunodeficiency virus (HIV) engaging in High-Risk sexual behavior: real world data from Northern Greece, 2019–2022. *Microorganisms*. 2024;12(7):1256.
- Befekadu B, Shuremu M, Zewdie A. Seroprevalence of syphilis and its predictors among pregnant women in Buno Bedele zone, Southwest Ethiopia: a community-based cross-sectional study. *BMJ Open*. 2022;12(8):e063745.
- Bristow CC, Adu-Sarkodie Y, Ondondo RO, Bukusi EA, Dagnra CA, Oo KY, et al. Multisite laboratory evaluation of a dual human immunodeficiency virus (HIV)/Syphilis Point-of-Care rapid test for simultaneous detection of HIV and syphilis infection. *Open Forum Infect Dis*. 2014;1(1):ofu015.
- Samje M, Fondoh VN, Nguefack-Tsague G, Kamalieu K, Mbanya D, Murphy EL, et al. Trends in serological markers of transfusion transmissible infections in blood donations at the Bamenda Hospital-based blood Service, Cameroon. *Transfus Clin Biol*. 2021;28(3):228–33.
- Mbanya MG, Longdoh NA, Nguoukam H, Ako SE. Syphilis and HIV infection among pregnant women previously screened negative during their first antenatal care visit (ANC) at some selected health facilities in the Buea health District, Cameroon. *J Biosci Med*. 2023;11(7):50–65.
- Grillo M, Tran BR, Tamoufe U, Djoko CF, Saylor K, Woodland K, et al. HIV and syphilis prevalence and associated risks in the Cameroonian armed forces. *Curr HIV Res*. 2017;15(2):137–45.
- Sama CB, Feteu VF, Tindong M, Tanyi JT, Bihle NM, Iii FFA. Prevalence of maternal HIV infection and knowledge on mother-to-child transmission of HIV and its prevention among antenatal care attendees in a rural area in Northwest Cameroon. *PLoS ONE*. 2017;12(2):e0172102.
- Zoufaly A, Onyoh EF, Tih PM, Awason CN, Feldt T. High prevalence of hepatitis B and syphilis co-infections among HIV patients initiating anti-retroviral therapy in the north-west region of Cameroon. *Int J STD AIDS*. 2012;23(6):435–8. Available at: <https://doi.org/10.1258/ijsa.2011.011279>
- Bissek ACZK. Cameroon population-based HIV impact assessment CAMPHIA 2017–2018. Available at: <https://stacks.cdc.gov/view/cdc/120052>
- Kengne-Nde C, Anoubissi J, de Loni-Ekali D, Nguéfeu-Nkenfou G, Moussa C, Messeh Y. Highlighting a population-based re-emergence of syphilis infection and assessing associated risk factors among pregnant women in Cameroon: evidence from the 2009, 2012 and 2017 National Sentinel surveillance surveys of HIV and syphilis. *PLoS ONE*. 2020;15(11):e0241999.
- Alere Inc. Alere's, SD BIOLINE HIV/Syphilis Duo is first dual test to receive WHO prequalification [press release]. PR Newswire; 2015. Available from: <https://www.prnewswire.com/news-releases/alere-sd-bioline-hiv-syphilis-duo-is-first-dual-test-to-receive-who-prequalification-300174380.html>
- Évaluation des performances et de la faisabilité du test combiné de dépistage du VIH/syphilis (SD Bioline HIV/syphilis Duo) chez les femmes enceintes à Yaoundé et Douala au Cameroun | Centre de Documentation Numérique du Secteur Santé. Available at: <https://cdnss.minsante.cm/?q=en/content/C3%A9valuation-des-performances-et-de-la-faisabilit%C3%A9-d-u-test-combin%C3%A9-de-d%C3%A9pistage-du-0>. Accessed.
- Omoding D, Katawera V, Siedner M, Boum Y. Evaluation of the SD bioline HIV/Syphilis duo assay at a rural health center in Southwestern Uganda. *BMC Res Notes*. 2014;7:746.
- Gliddon HD, Peeling RW, Kamb ML, Toskin I, Wi TE, Taylor MM. A systematic review and meta-analysis of studies evaluating the performance and operational characteristics of dual point-of-care tests for HIV and syphilis. *Sex Transm Infect*. 2017;93(5):S3–15. Available at: <https://sti.bmj.com/content/93/5/S3>. Accessed: 20 May 2023.
- Lodiongo DK, K Bior B, Dumo W, Katoro GS, Mogga J, Lokore J. Field evaluation of SD BIOLINE HIV/Syphilis duo assay among pregnant women attending routine antenatal care in Juba, South Sudan. *PLoS ONE*. 2018;13(10):e0205383.
- Shimelis T, Lemma K, Ambachew H, Tadesse E. Syphilis among people with HIV infection in Southern Ethiopia: sero-prevalence and risk factors. *BMC Infectious Diseases*. 2015;15(1):189. Available at: <https://doi.org/10.1186/s12879-015-0919-7>. Accessed: 21st April 2024.
- Uakarn C, Chaokromthong K, Sintao N. Sample Size Estimation using Yamane and Cochran and Krejcie and Morgan and Green Formulas and Cohen statistical power analysis by g\*power and comparisons. *APHEIT Int J Interdiscip Soc Sci Technol*. 2021;10(2):76–86. Available at: <https://so04.tci-thaijo.org/index.php/ATI/article/view/254253>. Accessed: 20 Oct 2024.
- Home - Biorex Diagnostics - Primary Diagnostics Innovation. Available at: <http://biorexdiagnostics.com/>. Accessed: 16 March 2024.
- Diagnostic Test Calculator. Diagnostic Test Calculator. Available at: <https://ebm-tools.knowledgetranslation.net/calculator/diagnostic/>. Accessed: 1st July 2024.
- Schoonjans F. Oct MedCalc. MedCalc's Diagnostic test evaluation calculator. Available at: [https://www.medcalc.org/calc/diagnostic\\_test.php](https://www.medcalc.org/calc/diagnostic_test.php). Accessed: 10 2024.
- Shakya G, Singh DR, Ojha HC, Ojha CR, Mishra SK, Malla K, et al. Evaluation of SD bioline HIV/syphilis duo rapid test kits in Nepal. *BMC Infect Dis*. 2016;16(1):450.
- Taremwa IM, Twelwanike A, Mwambi B, Atuhairwe C. Laboratory assessment of SD bioline HIV/Syphilis duo kit among pregnant women attending antenatal clinic Mayuge health center III, East central Uganda. *BMC Res Notes*. 2019;12(1):238.
- Withers K, Bristow C, Nguyen M, Stafylis C, Giang LM, Klausner JD. A field evaluation of a rapid dual immunoassay for human immunodeficiency virus and syphilis antibodies, Hanoi, Vietnam. *Int J STD AIDS*. 2019;30(2):173–80.
- Olugbenga I, Taiwo O, Laverty M, Ngige E, Anyaike C, Bakare R et al. Clinic-based evaluation study of the diagnostic accuracy of a dual rapid test for the screening of HIV and syphilis in pregnant women in Nigeria. *PLOS ONE*. 2018;13(7):e0198698. Available at: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0198698>. Accessed: 29 Dec 2024.
- Chiappe MA, Lopez-Torres L, Carcamo C, Garcia P, Peeling R. P5.090 Evaluation of a double rapid test for syphilis and HIV: SD Bioline HIV/Syphilis Duo. *Sex Transm Infect*. 2013;89(Suppl 1):A363–A363. Available at: [https://sti.bmj.com/content/89/Suppl\\_1/A363.1](https://sti.bmj.com/content/89/Suppl_1/A363.1). Accessed: 28 Dec 2024.
- Holden J, Goheen J, Jett-Goheen M, Barnes M, Hsieh YH, Gaydos CA. An evaluation of the SD bioline HIV/syphilis duo test. *Int J STD AIDS*. 2018;29(1):57–62.

## Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.