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Assessing the diagnostic accuracy of the SD-Ov16 Rapid Diagnostic Test compared to qPCR assay for the diagnosis of onchocerciasis in high and low endemic settings of Ghana

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Abstract

Background

Accurate diagnostic tools are essential for onchocerciasis elimination programs. The SD-Bioline Ov-16 rapid diagnostic test (RDT) has been widely adopted for field surveillance, but its performance compared to molecular methods requires evaluation. This study assesses the concordance between SD-Bioline Ov-16 RDT and quantitative polymerase chain reaction (qPCR) assay for diagnosing onchocerciasis in some endemic communities in Ghana.

Methods

A community-based cross-sectional study was conducted between September and November 2023 in seven communities across three districts in the Volta and Oti Regions of Ghana. Participants aged ≥ 5 years were recruited using convenience sampling. Dried blood spots were tested using the SD-Bioline Ov-16 RDT, and skin snips were analysed by qPCR targeting the O-150 repeat sequence both from same individuals. Concordance was assessed using kappa statistics, and diagnostic performance was estimated with qPCR as the reference standard.

Results

Out of 1,705 participants recruited, 902 with complete paired samples were analysed. The overall prevalence was 27.5% (248/902) by Ov-16 RDT and 2.9% (26/902) by qPCR. The highest prevalence was observed in Nkwanta North District (44.6% by RDT, 5.5% by qPCR). Overall concordance between tests was 73.4% with a kappa score of 0.0759 ($p < 0.001$), indicating poor agreement. The Ov-16 RDT showed 65.4% sensitivity (95% CI: 44.3–82.8%) and 73.6% specificity (95% CI: 70.5–76.6%) when compared to qPCR.

Conclusion

Poor concordance between Ov-16 RDT and qPCR reflects the fundamental difference between antibody detection (exposure) and parasite detection (active infection). While RDT remains useful for surveillance, its limitations in detecting active low-level infections necessitate improved diagnostic tools to support WHO's 2030 elimination goals. The persistent transmission in Nkwanta North District requires intensified intervention strategies.

Introduction

Human onchocerciasis, commonly known as river blindness, is a vector-borne neglected tropical disease caused by the filarial nematode *Onchocerca volvulus*. The parasite is transmitted through repeated bites

of infected blackflies of the genus *Simulium* (1). Approximately 99% of the estimated 21 million people infected with *O. volvulus* reside in Africa, where the disease poses significant public health and socioeconomic challenges (2). Clinical manifestations include severe pruritus, dermatitis, skin depigmentation, and ocular lesions that can progress to visual impairment and irreversible blindness. These pathological changes result from host inflammatory and immunological responses to dead or dying microfilariae (3).

Regional control efforts in Africa began with the Onchocerciasis Control Programme (OCP) in 1974, which initially involved 11 West African countries and relied primarily on vector control using larvicides. Subsequently, the African Programme for Onchocerciasis Control (APOC) was established in 1995, expanding coverage to 19 endemic countries and shifting focus to mass drug administration (MDA) with ivermectin through community-directed treatment strategies (4). Building on these successes, the World Health Organization (WHO) has transitioned from disease control to elimination, setting ambitious targets for interruption of transmission by 2030 (33). In 2016, WHO recommended the Ov-16 antibody-based serological test for mapping onchocerciasis endemicity and determining MDA eligibility, particularly in areas of low transmission intensity (1).

Achieving elimination requires robust diagnostic tools capable of detecting low-level infections and distinguishing active transmission from residual exposure. Traditionally, microscopic examination of skin snips has served as the gold standard for diagnosis and surveillance. However, this method has significant limitations: low sensitivity for detecting microfilariae at low parasite densities, invasiveness leading to community resistance, and concerns about transmission of blood-borne pathogens (5). These drawbacks prompted the development of antibody-based assays, initially using enzyme-linked immunosorbent assays (ELISA) to detect IgG4 antibodies against the Ov-16 antigen. While effective for monitoring and evaluation, ELISA testing requires laboratory infrastructure, standardized protocols, and resources that limit its field applicability, particularly in remote endemic areas (6).

The introduction of the SD-Bioline Ov-16 rapid diagnostic test (RDT) represented a significant advancement for field-based surveillance programs. This point-of-care test has been instrumental in mapping exercises and has contributed to verification of elimination in the Americas (3, 5). Despite widespread adoption, published data on the current performance characteristics of the SD-Bioline Ov-16 RDT remain limited. WHO guidelines explicitly recommend performance evaluation of RDTs prior to their use in stopping MDA assessments, yet comprehensive validation studies in diverse endemic settings are lacking (5, 8).

In contrast, quantitative polymerase chain reaction (qPCR) targeting the *O. volvulus* O-150 repeat sequence has demonstrated superior sensitivity for detecting parasite DNA in skin samples compared to microscopy (7, 18). By detecting parasite genetic material, qPCR provides evidence of active infection with viable parasites, making it particularly valuable for assessing ongoing transmission in low-prevalence settings approaching elimination thresholds (5, 18).

Given the critical need for reliable diagnostics in elimination programs and the paucity of data on RDT performance, this study aimed to assess the concordance between SD-Bioline Ov-16 RDT and qPCR assay for onchocerciasis diagnosis in endemic communities of Ghana. Specific objectives were to: determine the prevalence of onchocerciasis using both diagnostic methods in selected communities, evaluate the level of agreement between Ov-16 RDT and qPCR, and assess the diagnostic performance characteristics (sensitivity and specificity) of the Ov-16 RDT using qPCR as the reference standard. This study provides current data on Ov-16 RDT performance and contributes evidence to inform diagnostic strategies for onchocerciasis elimination efforts in Ghana and similar endemic settings.

Methods

Study Design and Setting

This community-based cross-sectional study was conducted between September-October 2023 in seven communities across three districts in Ghana: Nkwanta North District (Oti Region), Adaklu District (Volta Region), and Hohoe District (Volta Region). Communities were purposively selected based on historical endemicity data, categorizing them as either high or low transmission areas.

Nkwanta North District is in the Oti Region, approximately 554.4 km from Accra. Three communities were selected: Abunyanya, Gborsike, and Lancha. The district experiences distinct rainy (April-October) and dry (November-March) seasons, with mean annual rainfall ranging from 922-1,874 mm and relative humidity around 80%. Mean annual temperatures range from 11–39°C. Vegetation is characterized by forest-savannah transition woodland. This district is known to be an area of high endemicity for onchocerciasis and has received 24 rounds of MDA with ivermectin. (32). The therapeutic coverage reported since 2014 ranged from 78.29% to 96.13%, and the epidemiological coverages ranged from 68.19% to 96.13%. The reported epidemiologic and therapeutic coverage at the time of the study was 77.65% and 86.67% respectively. Adaklu District is situated in the Volta Region, approximately 198.7 km from Accra. Three communities were included: Hlihave, Afeyeame, and Dawanu. Annual rainfall ranges from 513.9-1,099.88 mm, with temperatures between 12–32°C. The vegetation is predominantly savannah woodland and grassland. Adaklu District is known to be a very low to non-endemic area and has received MDA only once in 2019 with therapeutic and epidemiologic coverages of 86.71% and 23.61% respectively (32). Hohoe District contributed one additional community, Abledzie, to compensate for lower-than-expected participation in other areas. The district is classified as a low endemic area and is yet to receive MDA for ivermectin (32). The predominant ethnic groups in these areas include Konkombas, Akans, and Ewes, with Christianity and Islam as the major religions. Twi and Ewe served as the official working language for the study.

Sample size calculation

Sample size was calculated using Sealed Envelope Ltd 2012 power calculator for binary outcome non-inferiority trials. A minimum of 283 participants per community was required, targeting a total of 1,705 participants.

Participant recruitment

Participants were recruited using convenience sampling, whereby eligible community members who presented themselves at survey sites and expressed willingness to participate were enrolled. Survey teams spent up to 5 days in each community to maximize recruitment. In communities where target enrolment was not achieved, recruitment extended to neighbouring communities. The survey team identified eligible individuals (aged ≥ 5 years) and explained the study purpose, procedures, risks, and benefits. Demographic information was obtained from them using structured questionnaires administered on smartphones/tablets via the RedCap application.

Sample collection

Preparation of dried blood spots

Venous blood (5 ml) was collected from each participant into EDTA tubes. Dried blood spots (DBS) were prepared following standard operating procedures for SD-Bioline Ov-16 RDT testing (34). Two TropBio filter papers, each with six disk extensions, were used per participant, yielding 12 DBS per individual. Each disk was spotted with 10 µl of whole blood, air-dried, and stored at -20°C until laboratory testing.

Skin snipping

Skin snip biopsies were obtained from the iliac crest of participants 18 years and above, using sterile techniques (35). Samples were immediately placed in 70% ethanol and stored at -20°C pending qPCR analysis.

Laboratory analysis

Screening with RDT

Laboratory-based testing was performed on DBS samples using SD-Bioline Onchocerciasis IgG4 RDT kits (Standard Diagnostics Inc., Republic of Korea) according to manufacturer instructions (34). Tests were read and interpreted by trained personnel after the specified incubation period. Results were recorded as positive (presence of both control and test lines) or negative (control line only).

Processing of skin snips & DNA extraction

Ethanol preserved skin snips were centrifuged to remove the ethanol, leaving any sediments at the bottom of the tube. The sediment was washed with PBS-Tween by a series of centrifugation and

discarding of filtrate. Following the manufacturer's protocol DNA was extracted from sediments using the Qiagen Investigator kit after 10µl of *Bacillus atrophaeus* control (BAC) was added to validate the nucleic acid extraction procedure. Briefly, 180µl and 20µl of Buffer ATL and proteinase K was added respectively, following a 56℃ overnight incubation at 600rpm. 200µl of Buffer AL, followed by 200µl of molecular grade ethanol was added, pulse vortexed and incubated for 5 minutes at room temperature. Entire lysate was then transferred to the QiAamp MinElute column, followed by 500µl Of Buffer AW1, 600µL of Buffer AW2, 500µl of molecular grade ethanol, with each step centrifuged at 8000rpm for 1 minute. 50µl of elution buffer, ATE was added to the membrane and incubated at room temperature for 20 minutes and centrifuged at 14,000 rpm for 1 minute.

Detection of O. volvulus using qPCR

A TaqMan-based qPCR assay targeting the *O. volvulus* O-150 repeat sequence (31) was performed using a QuantStudio 5.0 Real-Time PCR system (Thermo Scientific). Each 10 µl reaction contained: 5.0 µl TaqMan Fast Universal Master Mix, 0.3 µl forward primer (O-150F), 0.3 µl reverse primer (O-150R), 0.25 µl TaqMan probe, 2.15 µl nuclease-free water, and 2.0 µl template DNA. Cycling conditions were initial denaturation at 95°C for 20 seconds, followed by 40 cycles of denaturation at 95°C for 1 second and annealing/extension at 57°C for 30 seconds. Plasmid controls BAC was used as the DNA extraction internal control and OvPC (*O. volvulus* positive control) was used as the reference positive control for detecting *O. volvulus* presence. Samples with Ct values ≤ 35 were classified as positive for *O. volvulus* DNA.

Data Management and Statistical Analysis

Data were collected electronically using RedCap and exported to Microsoft Excel 2007 for preliminary cleaning and compilation. Statistical analyses were performed using R software (version 4.3.3). Data collectors received training on study objectives, questionnaire administration, and interview techniques prior to fieldwork. The principal investigators supervised data collection activities and performed quality checks for completeness and consistency. Descriptive statistics were performed for demographic and clinical variables. Categorical variables are presented as frequencies and percentages. The prevalence of onchocerciasis was estimated for both diagnostic methods for the overall and then stratified by district, community, age group, sex, duration of residence, and ivermectin treatment history.

Agreement between SD-Bioline Ov-16 RDT and qPCR was assessed using Cohen's kappa statistic with 95% confidence intervals. Kappa values were interpreted as follows: <0 (no agreement), 0-0.20 (poor), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (substantial), and 0.81-1.00 (almost perfect agreement). Using qPCR as the reference standard, diagnostic performance characteristics of the Ov-16 RDT were calculated including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), along with their 95% confidence intervals using the Clopper-Pearson method P-values < 0.05 were considered statistically significant.

Results

Study Population and Participant Characteristics

A total of 1,705 participants were initially recruited across the seven study communities. Of these, 902 adults who provided both skin snip specimens and dried blood spots formed the final analytical sample for comparative analysis. Most of these participants were female (558, 61.9%), while 344 (38.1%) were male (Table 1). Age distribution showed that more than half of participants (53.7%) were between 31–60 years, followed by those aged 15–30 years (33.4%), those > 60 years (12.9%), and only 1 (0.1%) participant aged 5–14 years. Regarding duration of residence in the study communities, most participants (62.6%) had lived there since birth, while 21.8% reported residing in the community for more than 10 years. Smaller proportions had lived in the community for 5–10 years (7.3%), 1–5 years (7.0%), or less than one year (1.2%) (Table 1).

With respect to ivermectin treatment history, 464 participants (51.4%) reported having previously received ivermectin for onchocerciasis, while 216 (23.9%) stated they had not received treatment (Table 1). Four participants (0.4%) could not remember their treatment history. Notably, 218 participants (24.2%) did not provide information regarding ivermectin treatment history.

Table 1
Sociodemographic characteristics and ivermectin treatment history of study participants (N = 902)

Variable	N	Percentage (%)
Age group (Years)		
5–14	1	0.1
15–30	301	33.4
31–60	484	53.7
> 60	116	12.9
Sex		
Female	558	61.9
Male	344	38.1
Duration of residence in community		
< i year	11	1.2
1–5 years	63	7.0
6–10 years	66	7.3
> 10 years	197	21.8
Since birth	565	62.6
Ivermectin treatment history		
Yes	464	51.4
No	216	23.9
Don't remember	4	0.4
No response	218	24.2

Prevalence by Sociodemographic Characteristics

By age group, Ov-16 RDT positivity was highest among participants aged 31–60 years (31.6%), followed by those > 60 years (25.9%), those aged 15–30 years (21.6%), and the single participant aged 5–14 years (0.0%) (Table 3). For qPCR, the highest prevalence was observed in the 15–30 age group (4.0%), followed by those aged 31–60 years (2.5%) and > 60 years (1.7%).

Stratification by sex revealed that males had higher prevalence than females for both diagnostic methods (Table 3). Using RDT, male prevalence was 29.4% (101/344) compared to 26.3% (147/558) for

females. Similarly, qPCR detected infection in 3.2% of males versus 2.7% of females.

When analysed by duration of residence, participants who had lived in the community for more than 10 years showed the highest RDT prevalence (39.1%), followed by those living there since birth (26.9%) and those with less than one year of residence (27.3%) (Table 3). For qPCR, the highest prevalence was found among participants residing less than one year (9.1%), followed by those living there since birth (3.5%) (Table 2).

Regarding ivermectin treatment history, among those who responded to this question, participants reporting previous ivermectin treatment showed RDT prevalence of 41.4% and qPCR prevalence of 3.9% (Table 3). Those who had not received treatment showed lower RDT prevalence (19.0%) but similar qPCR prevalence (3.2%) (Table 2).

Table 2
Prevalence of onchocerciasis by sociodemographic characteristics

Variable	Total	RDT Positive (%; 95% CI)	qPCR Positive (%; 95% CI)
Age Group (Years)			
5–14	1	0 (0.0; 0–95)	0 (0.0;0–95)
15–30	301	65 (21.6;17–27)	12 (4.0;2.2-7)
31–60	484	153 (31.6; 28–36)	12 (2.5;1.3–4.4)
> 60	116	30 (25.9; 18–35)	2 (1.7; 0.3–6.7)
Sex			
Female	558	147 (26.3; 23–30)	15 (2.7; 1.6–4.5)
Male	344	101 (29.4; 25–35)	11 (3.2; 1.7–5.8)
Duration Of Residence			
Below One Year	11	3 (27.3; 7.3–61)	1 (9.1; 0.48-43)
Between 1–5 Years	63	8 (12.7; 6–24)	1 (1.6; 0.08–9.7)
Between 5–10 Years	66	8 (12.1; 5.7–23)	0 (0.0; 0-6.9)
More Than 10 Years	197	77 (39.1; 32–46)	4 (2.0; 0.65–5.5)
Since Birth	565	152 (26.9; 23–31)	20 (3.5; 2.2–5.5)
Ivermectin Treatment			
Yes	464	192 (41.4; 37–46)	18 (3.9; 2.4–6.2)
No	216	41 (19.0; 14–25)	7 (3.2; 1.4–6.8)
Don't Remember	4	2 (50.0; 15–85)	0 (0.0; 0–60)
No Response	218	13(6.0;2.9–9.1)	1(0.5;0.01–2.5)

Prevalence by District and Community

Nkwanta North District recorded the highest number of participants (455) and demonstrated markedly elevated prevalence by both methods: 44.6% (95% CI: 40.0-49.3%) by RDT and 5.5% (95% CI: 3.6-8.0%) by qPCR (Table 4). Adaklu District had 345 participants with low prevalence by both methods: 5.8% (95%CI: 3.7%-9%) by RDT and 0.3% (95%CI: 0.02%-1.9%) by qPCR. Hohoe District, with 102 participants from a single community, showed intermediate RDT prevalence of 24.5% (95%CI:17%-35%,) and no qPCR-positive cases (Table 3).

At the community level, Abunyanya in Nkwanta North District had the largest sample (347 participants) and the highest prevalence (53.6%) by RDT and 7.2% by qPCR (Table 4). Other communities in Nkwanta North (Lancha and Gborsike) also showed elevated RDT prevalence (16.5% and 11.8%, respectively), although no qPCR-positive cases were detected. The three communities in Adaklu District (Hlihave, Afeyeame, Dawanu) all demonstrated low prevalence by both methods, ranging from 4.9–7.6% by RDT and 0-0.6% by qPCR. Abledzie (Hohoe District) showed 24.5% RDT prevalence with no qPCR-positive cases (Table 3). All three districts demonstrated poor agreement (kappa < 0.20). Nkwanta North District showed the lowest agreement (57.4%, kappa = 0.0568, p = 0.016), while Adaklu District had the highest agreement (93.9%, kappa=-0.0056, p = 0.804).

Table 3
Prevalence of onchocerciasis by district and community

Site	RDT Positive (%; 95% CI)	qPCR Positive (%; 95% CI)	% Agreement (Kappa 95% CI)	Total
Nkwanta North District				
Abunyanya	186 (53.6;48–59)	25 (7.2;4.8–11)		347
Lancha	15 (16.5;9.8–26)	0 (0.0;0.0–5.0)		91
Gborsike	2 (11.8;2.1–38)	0 (0.0;0.0–23)		17
Overall	**203 (44.6;40–49)**	**25 (5.5;3.7–8.1)**	57.4 (0.0568) *	**455**
Adaklu District				
Hlihave	8 (4.9;2.3–9.8)	1 (0.6;0.03–3.9)		162
Afeyeame	4 (5.1;1.7–13)	0 (0.0;0.0-5.8)		78
Dawanu	8 (7.6;3.6–15)	0 (0.0;0.0-4.4)		105
Overall	**20 (5.8;3.7-9.0)**	**1 (0.3;0.02–1.9)**	93.9 (-0.0056) *	**345**
Hohoe District				
Abledzie	25(24.5;17–34)	0(0.0;0.0-4.5)	75.5 (0.0000) *	102

% = Percentage of positive individuals

CI = Confidence interval

*Statistically significant difference ($p < 0.001$)

Concordance Between Ov-16 RDT and qPCR

Out of the 902 participants with paired results for both RDT and qPCR, 645 (71.5%) tested negative by both methods, representing true concordant negatives (Table 4). Only 17 participants (1.9%) tested

positive by both methods (concordant positives). Discordant results were observed in 240 participants (26.6%). Most of the discordance was RDT-positive/qPCR-negative (231 participants, 25.6%), while 9 participants (1.0%) were RDT-negative/qPCR-positive (Table 5). The overall percentage agreement between the two tests was 73.4% (95% CI: 70.4–76.2%). Cohen's kappa coefficient was 0.0759 (95% CI: 0.0089–0.1428), indicating poor agreement between the tests. This low kappa value reflects substantial disagreement beyond what would be expected by chance alone.

Using qPCR as the reference standard for active infection, the diagnostic performance characteristics of the Ov-16 RDT were calculated. The sensitivity was 65.4% (95% CI: 44.3–82.8%). The specificity was 73.6% (95% CI: 70.5–76.6%). The positive predictive value was 6.9%, (95% CI: 4.1–10.7%). The negative predictive value was 98.6% (95% CI: 97.5–99.3%).

Table 4
Cross-tabulation of SD-Bioline Ov-16 RDT and qPCR results

	RDT RESULTS		
qPCR Result	**Negative N (%)**	**Positive N (%)**	**Total N (%)**
Negative	645 (71.5)	231 (25.6)	876 (97.1)
Positive	9 (1.0)	17 (1.9)	26 (2.9)
Total	**654 (72.5)**	**248 (27.5)**	902 **(100)**

% = percentage

N= Number examined

Table 5
Diagnostic performance of Ov-16 RDT in two endemicity settings

Endemic districts	**sensitivity (%)**	**specificity (%)**	**KAPPA(P-Value)**
Low (ADAKLU)	0 (0.0-97.5)	94.2 (91.2—96.4)	-0.0056 (0.8038)
High (NKWANTA & hohoe)	68.0 (46.5–85.1)	60.3 (56.0-64.5)	0.0582 (0.0049)

A test performance matrix based on the sensitivity and specificity of the Ov-16 RDT in endemic areas was also conducted. Adaklu District representing the low endemic area recorded a specificity of 94% and a sensitivity of 0%. Nkwanta and Hohoe districts representing the highly endemic areas recorded a sensitivity and specificity of 68.0% and 60.3% respectively. The kappa values were − 0.0056 (p = 0.804) and 0.0582 (p = 0.0049) for the low endemic district and high endemic districts, respectively.

Discussion

This study assessed the concordance between SD-Bioline Ov-16 RDT and qPCR for onchocerciasis diagnosis in endemic communities of Ghana. Our findings reveal substantial discordance between these two diagnostic methods, with important implications for elimination programs. The overall prevalence was 27.5% by Ov-16 RDT and 2.9% by qPCR, representing nearly a 10-fold difference. The RDT demonstrated 65.4% sensitivity and 73.6% specificity when compared to qPCR as the reference standard.

The markedly higher prevalence detected by Ov-16 RDT compared to qPCR reflects the fundamental biological difference between what these tests measure. The Ov-16 RDT detects IgG4 antibodies against the Ov-16 antigen, which indicate immunological exposure to *O. volvulus* infection (19, 20). These antibodies can persist for years following exposure, even after successful treatment or natural parasite death (7, 16, 24). In contrast, qPCR detects parasite DNA in skin tissue, providing direct evidence of current infection with viable or recently dead parasites (18).

The 27.5% RDT prevalence likely represents cumulative exposure in these endemic communities, encompassing individuals with current active infections, past resolved infections, and those who have been successfully treated. The 2.9% qPCR prevalence more accurately reflects ongoing active transmission, although even this may underestimate true prevalence due to sampling limitations (only two skin snips from one body site) and possible low parasite densities below the detection threshold (18).

The geographic distribution of infections is particularly concerning. Nkwanta North district demonstrated alarmingly high prevalence by both methods (44.6% RDT, 5.5% qPCR), with Abunyanya community showing even higher rates (53.6% RDT, 7.2% qPCR). This 5.5% qPCR prevalence far exceeds WHO's elimination threshold of < 0.1% for endemic populations and indicates substantial ongoing transmission despite years of MDA implementation (8, 10). These findings suggest that current interventions in Nkwanta North are insufficient to interrupt transmission, although the ESPEN data (32) records 24 rounds of MDA delivered, there is a need for intensified strategies such as increased MDA coverage, improved treatment compliance, potential biannual distribution, and vector control measures (11, 12, 28).

In contrast, Adaklu district showed very low prevalence by both methods (5.8% RDT, 0.3% qPCR). According to the ESPEN data, Adaklu is described as a low to non- endemic area with mass drug administration not delivered (32). Among the 345 participants tested in Adaklu, those who had resided in the community since birth accounted for the highest proportion of positive cases (4.3%, 95% CI:2.5–7.2, n = 15). This shows that despite the low endemicity of the area, there is local transmission of the parasite which could be as a result of migration to highly endemic areas. Conversely, the lowest prevalence was observed among individuals who had lived in the district for only one to five years (0.3%, 95% CI:0.0-1.9, n = 1). This may be recent infections acquired by individuals. Hence there is a need for a sustained delivery of mass drug administration in this area in order to control local transmission. (8, 10).

The higher prevalence in males compared to females (29.4% vs 26.3% by RDT; 3.2% vs 2.7% by qPCR) aligns with occupational exposure patterns documented in other studies (6, 27). Men in these communities typically engage in agricultural activities and spend more time outdoors near breeding sites of *Simulium* vectors, increasing their exposure risk (14, 15). Additionally, cultural practices regarding clothing may provide some protection for women, who often wear more covering garments during outdoor activities.

The age distribution of infections reveals important epidemiological patterns. The highest RDT prevalence in the 31–60 age group (31.6%) likely reflects cumulative exposure over decades of living in endemic areas, resulting in sustained antibody responses (7, 24). However, the highest qPCR prevalence in the 15–30 age group (4.0%) is particularly concerning from an elimination perspective. Active infections in young adults may suggest recent transmission and ongoing parasite circulation, as this cohort has lived their entire lives during the MDA era. Also, they are the actively working group likely to be involved in farming or other outdoor activities which get them more exposed to bites. (10, 28). This pattern may indicate: (i) suboptimal MDA coverage or treatment compliance in younger populations, (ii) continued high-level transmission overwhelming the impact of annual treatment, or (iii) behavioural factors placing young adults at higher risk of blackfly exposure (13, 29).

The poor concordance (kappa = 0.0759) between Ov-16 RDT and qPCR was expected given the different biological markers detected, but the magnitude of discordance has important programmatic implications (5, 6, 24). The large number of RDT-positive/qPCR-negative participants (representing 25.6% of all samples) likely represents individuals with past exposure but no current detectable infection (16, 17). This could include: (i) successfully treated individuals with persistent antibodies, (ii) individuals who spontaneously cleared infections, or (iii) those with infections below the qPCR detection threshold or not represented in the single skin snip obtained (18, 24).

More concerning are the 9 participants (1.0%) who were RDT-negative but qPCR-positive. These false-negative RDT results indicate individuals with active infections who would be missed by serological screening (22, 25). Possible explanations include: (i) very recent infections where antibody responses have not yet developed (though Ov-16 antibodies typically appear within months) (19, 24), (ii) immunological non-responders who fail to mount detectable antibody responses, or (iii) technical issues with RDT performance or storage (22, 25).

The 65.4% sensitivity of Ov-16 RDT observed in our study falls substantially below WHO's recommended minimum of 89% for stopping MDA assessments and even below the 60% minimum for elimination mapping (8). This contrasts sharply with the 91.3% sensitivity reported by Ekanya *et al*. in Cameroon (6). Several factors may explain this discrepancy. First, our study used skin snip qPCR as the reference standard, which detects active infection, while Ekanya *et al*. compared RDT to Ov-16 ELISA, which also measures antibodies and would be expected to show higher concordance with RDT (6, 19). Second, differences in transmission intensity, MDA history, and population exposure patterns between study

settings may affect test performance (7, 24, 25). Third, our sample included predominantly older adults with longer residence, potentially affecting the sensitivity estimates.

The 73.6% specificity also falls far short of WHO's required ≥ 99.8% for elimination assessments (8). In practical terms, this means that in low-prevalence settings approaching elimination (e.g., 1–2% true prevalence), the RDT would generate substantial false-positive results, potentially leading to inappropriate continuation of MDA or misclassification of elimination status (9). The positive predictive value of only 6.9% indicates that fewer than 1 in 14 RDT-positive results represent true active infections, severely limiting the test's utility for identifying infected individuals.

Conversely, the high negative predictive value (98.6%) suggests that RDT-negative results reliably exclude infection, making the test more useful for ruling out rather than ruling in disease (5, 25). However, even this relatively high NPV is problematic in elimination settings where missing even rare infections could allow continued transmission (8, 10).

WHO's target product profiles for onchocerciasis diagnostics specify stringent performance criteria based on programmatic needs (8). For elimination mapping, sensitivity ≥ 60% and specificity ≥ 99.8% are required. For stopping MDA assessments, even higher sensitivity (≥ 89%) with the same specificity (≥ 99.8%) is mandated. These stringent requirements aim to: (1) detect true low-level transmission to prevent premature cessation of interventions, (2) minimize false positives that would unnecessarily continue costly MDA programs, and (3) provide confidence in elimination verification decisions affecting millions of people.

The sensitivity and specificity of 0% and 94% recorded in low endemic areas indicate that the RDT may not be useful to the elimination program when considering low endemic areas and elimination settings. This means that positive individuals would go undetected, thereby affecting treatment decisions and hence affecting WHO decisions to stop MDA, as a less sensitive tool means the areas evaluated would fall below the prevalence-stopping MDA threshold (36). The RDT recorded a sensitivity of 68% in high endemic areas, implying its usefulness in detecting infected individuals' areas in need of mapping or continued surveillance. However, a low specificity of 60% was recorded in high endemic areas. This raises concerns about the generation of a lot of false positive results in high endemic areas, thereby leading to the error of continuous treatment (37). These findings indicate that in a low endemic area a more sensitive than specific tool is needed to make out infected individuals to treat and a more specific than sensitive tool is needed in high endemic areas in order to inform treatment decisions to stop or continue MDA.

Our findings indicate the SD-Bioline Ov-16 RDT does not meet these criteria when validated against qPCR, particularly falling short on specificity (5, 8, 9). This has significant implications for elimination programs relying primarily on RDT for decision-making. The test may be adequate for initial endemicity mapping in previously unmapped areas or for detecting high-level transmission (16, 23) but appears unsuitable as a standalone tool for stopping MDA or verifying elimination (5, 8).

Several limitations must be acknowledged. First, the convenience sampling approach may introduce selection bias, as participants who presented for testing might differ systematically from non-participants in exposure or infection risk (13). Second, the cross-sectional design provides only a snapshot of infection status; longitudinal follow-up would better elucidate antibody kinetics and infection dynamics (7, 24). Third, we collected only two skin snips from one body site (iliac crest; one on each side), which may underestimate true infection prevalence as microfilariae distribution can be patchy and vary by body location (18). Finally, the small number of qPCR-positive cases (n = 26) results in wide confidence intervals for sensitivity estimates, limiting precision of performance calculations (25).

Conclusion

This study revealed poor concordance between SD-Bioline Ov-16 RDT and qPCR for onchocerciasis diagnosis, with overall agreement of 73.4% and kappa of 0.0759. While this discordance largely reflects the fundamental difference between antibody-based detection of exposure versus molecular detection of active infection, the RDT's diagnostic performance (65.4% sensitivity, 73.6% specificity) falls substantially below WHO targets for elimination program decision-making. The persistently high prevalence in Nkwanta North District indicates ongoing transmission far exceeding elimination thresholds, necessitating urgent intensification of control measures. Conversely, low prevalence in Adaklu District suggests progress toward elimination but requires continued surveillance. The Ov-16 RDT remains valuable for rapid field-based surveillance and initial endemicity mapping, particularly in resource-limited settings. Achieving WHO's 2030 elimination goals will require not only sustained implementation of effective interventions but also improved diagnostic tools capable of reliably detecting the low-level infections that characterize late-stage elimination settings.

Abbreviations

MDA
Mass Drug Administration
RDT
Rapid Diagnostic Test
qPCR
quantitative Polymerase Chain Reaction
ESPEN
Expanded Special Project for Elimination of Neglected Tropical Diseases
WHO
World Health Organization
ELISA
Enzyme Linked Immunosorbent Assay
APOC
African Programme for Onchocerciasis Control
OCP

Onchocerciasis Control Programme

BAC

Bacillus atrophaeus Control

NPV

Negative Predictive Value

PPV

Positive Predictive Value

EDTA

Ethylenediaminetetraacetic acid

DNA

Deoxyribonucleic acid

DBS

Dried Blood Spot

OvPC

Onchocerca volvulus Positive Control

Declarations

Ethical Considerations

This study received ethical approval from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana (Protocol Number: 013/23–24). Written informed consent was obtained from all adult participants (≥ 18 years). For participants < 18 years, written parental consent and, when applicable (ages 12–17 years), child assent was obtained prior to enrolment. All participants were assigned unique identification codes to maintain confidentiality. Study findings and individual results were communicated to district health authorities and participants as appropriate.

Consent for publication

Not applicable

Competing Interests

The authors declare that they have no competing interests.

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Author Contribution

A.A.N, S.M.D, M.S.A, Y.A and D.K.d.S conceived the idea for the manuscript. A.A.N, S.M.D, M.S.A, G.O.G, KN acquired the data from the field and analyzed the samples. K.N analyzed the data. B.L handled the data management. A.A.N drafted the manuscript and Y.A, D.K.d.S and K.K.F reviewed the manuscript. All authors read and approved the final manuscript

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Data Availability

The datasets generated/ analyzed during the study are not publicly available due to privacy and ethical considerations but are available from the corresponding author on reasonable request.

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