

Quality Assessment of Malaria Microscopy in Ouagadougou Laboratories, Burkina Faso: Accuracy of Thick and Thin Blood Smears

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Abstract

Background

Malaria microscopy remains the reference method for parasite detection in many low-resource settings, but its accuracy depends on slide preparation, staining quality, and reader performance.

Objective

This study aimed to assess the accuracy of routine malaria microscopy in selected laboratories in Ouagadougou, Burkina Faso.

Methods

We conducted a cross-sectional study in four laboratories in Ouagadougou, Burkina Faso, and re-examined 1,000 malaria blood smears initially read by 29 laboratory providers using independent expert microscopy.

Results

Twenty-nine microscopy providers participated, of whom 79.3% were biomedical technicians, 17.2% trainees, and 3.4% biologists. Routine microscopy classified 184/1000 slides (18.4%) as positive and 816/1000 (81.6%) as negative, whereas expert re-reading identified 115/1000 (11.5%) as positive and 885/1000 (88.5%) as negative. Overall concordance was 88.1%, with a sensitivity of 78.3%, specificity of 89.4%, positive predictive value of 48.9%, and negative predictive value of 96.9%. Inaccurate routine readings were significantly associated with lack of refresher training and shorter slide-reading time.

Conclusion

Routine malaria microscopy performance in the participating laboratories was limited by false-positive readings, highlighting the need for strengthened quality assurance and continuous competency-based training.

1. Introduction

Malaria continues to pose a formidable global public health challenge, driven by *Plasmodium* parasites transmitted through *Anopheles* mosquitoes, with sub-Saharan Africa accounting for over 90% of cases and deaths [1]. The WHO's World Malaria Report 2025 underscores stagnant progress, with approximately 249 million cases and 608,000 deaths in 2024, urging renewed focus on diagnostics amid

emerging threats like insecticide resistance and climate impacts [1]. Burkina Faso exemplifies this burden, ranking among the top contributors; in 2023, it reported over 12 million cases and 4,000 deaths mostly in children under five straining health systems despite recent interventions like the End Malaria Council launched in 2025 [2].

Nationally, malaria dominates morbidity: it led consultations, hospitalizations, and mortality in 2016 (43%, 45%, and 22%, respectively), with 11 million uncomplicated cases and over 7,000 deaths by 2018. Recent data show a historic 32% case decline to 7.33 million and 48% mortality drop by 2025, yet urban hotspots like Ouagadougou persist amid high transmission [5]. *P. falciparum* prevails, necessitating precise diagnosis for severe case management and to curb overtreatment [3].

Microscopy of thick smears (for density) and thin films (for speciation) remains the gold standard, enabling qualitative and quantitative assessment with minimal equipment [4]. In Burkina Faso, over 250,000 thick smears were processed in 2016 alone, but accuracy depends on technician skill, staining quality, and quality control. False results positives or negatives distort epidemiology, fuel resistance, and impair care [6].

Recent studies (2018–2025) expose ongoing flaws. WHO-supported proficiency testing in the Democratic Republic of Congo (2021) found > 66% of density estimates erroneous, linked to poor Giemsa staining (< 20% compliant) and outdated training [22]. In Tanzania's military facilities (2016–2018), external quality assessment (EQA) improved detection from 60% to 90% via supervision, yet false negatives tied to preparation persisted [4]. Ethiopia's 2020 EQA revealed 97% detection concordance but inconsistent density reporting [6]. Regionally, a 2024 Senegal audit affirmed WHO/ISO 15189 compliance in a reference lab, stressing sustained microscopist training.

These findings reveal gaps: urban private labs, handling high volumes, are understudied versus rural sites. Low-density infections (< 500/μL) evade detection, skewing data, while technician profiles (technicians, trainees, biologists) correlate poorly with performance [4]. Post-2020 literature demands localized EQA integrating rechecking and on-site training to support elimination goals [1].

This cross-sectional study in Ouagadougou's four medical-surgical centers evaluates thick/thin smear quality via expert re-reading of 1,000 slides from 29 providers, addressing urban diagnostic voids.

The primary aim of this study was to assess the accuracy of malaria microscopy results in selected laboratories in Ouagadougou, Burkina Faso. These insights will enhance urban diagnostics, aligning with Burkina Faso's malaria reduction trajectory [5].

2. Methods

2.1. Study Design

This cross-sectional prospective study was conducted over five months, from July 13 to November 10, 2021, in Ouagadougou, Burkina Faso. It assessed malaria microscopy quality by comparing routine laboratory readings of thick and thin blood smears against expert re-readings [1].

2.2. Study Area

The investigation occurred in Ouagadougou, capital of Kadiogo province and Burkina Faso's largest city, home to approximately 2.9 million residents across 2,805 km² under a Sudano-Sahelian climate. Data collection targeted medical biology laboratories in four health centers spanning the city's five districts: Baskuy, Bogodogo, Boulmiougou, Sig-Nonghin, and Nongr-Massom.

2.3. Population and Sampling

The target population comprised thick smear and/or thin blood smear slides prepared and interpreted for malaria diagnosis by laboratory staff in public and private facilities. We purposively collected 1000 slides with their initial results, representing readings by 29 microscopy providers.

2.4. Inclusion and Exclusion Criteria

Inclusion criteria encompassed all thick smear alone or thick-plus-thin smear slides read during the study period with available results. Exclusion applied to defective slides (e.g., broken, faded, or unreadable) or those lacking documented outcomes.

2.5. Data Collection

A structured questionnaire captured provider socio-demographics, training, and practices (see Supplementary Questionnaire). Initial slide results positivity, *Plasmodium* species, and density were recorded on standardized forms at collection. Expert readers used confidential individual grids for re-reading results.

2.6. Laboratory and Analytical Methods

Post-routine analysis, slides were blindly re-examined independently by two senior biomedical technicians from the Yalgado Ouédraogo University Hospital (CHU-YO) parasitology service national reference for malaria microscopy and the principal investigator. The first expert had > 11 years' experience; the second, > 20 years. Concordance among at least two readers defined the gold standard for positivity, speciation, and density categories [4].

Key variables included:

- Provider profile: qualification (technician, trainee, biologist), experience, refresher training.
- Technical factors: standard operating procedures (availability/adherence), equipment/reagent status.
- Slide characteristics: preparation type, reading quality.

- Performance metrics (routine vs. reference): sensitivity, specificity, positive/negative predictive values (calculated per standard 2×2 contingency formulas) [7].
- Influencing factors (e.g., workload, staining) were noted for correlation analysis.

2.7. Statistical Analysis

Data underwent double-entry verification before analysis in Epi Info 3.5.4. Descriptive statistics summarized frequencies, percentages, and means. Associations between provider characteristics, facility type, and performance used chi-square or Fisher's exact tests (expected frequencies < 5). Statistical significance was set at $p < 0.05$. Odds ratios with 95% confidence intervals were calculated. Microsoft Excel 2016 generated tables [8]. Sensitivity, specificity, PPV and NPV were calculated using 2×2 contingency tables.

3. Results

3.1. Provider Characteristics

Twenty-nine microscopy providers from four health facilities participated (Table 1). Site 1 (n = 11, 37.9%) and Site 4 (n = 7, 24.1%) contributed the most providers, followed by Site 2 (n = 6, 20.7%) and Site 3 (n = 5, 17.2%). Biomedical technicians predominated (23/29, 79.3%), with trainees (5/29, 17.2%) and one biologist (3.4%) (Table 1). Experience exceeded 10 years for 15 providers (51.7%), while 6 (20.7%) had < 2 years (Table 1). Refresher training was reported by 12 (41.4%); 17 (58.6%) had none.

Table 1
Characteristics of participating health facilities and microscopy providers

Variable	Category	n (%)
Health facility	Site 1	11 (37.9)
	Site 2	6 (20.7)
	Site 3	5 (17.2)
	Site 4	7 (24.1)
Professional qualification	Biologist	1 (3.4)
	Biomedical technologist	23 (79.3)
	Trainee	5 (17.2)
Seniority × refresher training	< 2 years – with refresher training	0 (0.0)
	< 2 years – no refresher training	6 (20.7)
	2–5 years – with refresher training	1 (3.5)
	2–5 years – no refresher training	3 (10.3)
	5–10 years – with refresher training	1 (3.5)
	5–10 years – no refresher training	3 (10.3)
	> 10 years – with refresher training	10 (34.5)
	> 10 years – no refresher training	5 (17.2)
Availability of SOPs	Staining and smear preparation	4 (100.0)
	Reading and interpretation	3 (75.0)
	Stock Giemsa solution preparation	3 (75.0)
	Blood smear preparation (thick/thin)	3 (75.0)
	Microscope use, maintenance, and care	3 (75.0)
Distribution of study sites, professional qualifications, seniority and participation in refresher training, and availability of standard operating procedures (SOPs) for malaria microscopy.		

No facility demonstrated full adherence to standard operating procedures. All four possessed staining/preparation SOPs; three had guidance for reading/interpretation, Giemsa preparation, smear production, and microscope maintenance (Table 1). All used Giemsa stain, binocular microscopes, frosted slides, and protected reagents from heat/light.

3.2. Slide Characteristics and Routine Readings

Among 1,000 slides, 824 (82.4%) were thick smears alone; 176 (17.6%) combined thick/thin smears (Table 2). Routine readings deemed 816 (81.6%) negative and 184 (18.4%) positive. Site-specific positivity: Site 3 (79/250, 31.6%), Site 4 (56/250, 22.4%), Site 2 (41/250, 16.4%), Site 1 (8/250, 3.2%) (Table 3). Of positives, species identification occurred in 64 (34.8%), all *P. falciparum*. Density was quantified for 175, ranging 12–496,843 parasites/MI (mean 12,626/MI; SD 38,259); Site 1 showed lowest mean (260/MI) (Table 4).

Table 2
Slide characteristics and routine reading practices

Variable	Category	n (%)
Type of blood film	Thick smear only	824 (82.4)
	Thin smear only	0 (0.0)
	Thick plus thin smear	176 (17.6)
Daily workload	< 5 slides per day	2 (6.9)
	≥ 10 slides per day	27 (93.1)
Reading time per slide	< 10 minutes	14 (48.3)
	≥ 10 minutes	15 (51.7)
Fields examined per slide	< 100 fields	13 (44.8)
	≥ 100 fields	16 (55.2)
Routine species identification	Yes	9 (31.0)
	No	20 (69.0)
Types of blood films prepared, daily workload, reading time per slide, number of fields examined before declaring negativity, and routine species identification		

Table 3
Routine microscopy results by site

Site	Routine result	n (%)
Site 1	Negative	242 (96.8)
	Positive	8 (3.2)
Site 2	Negative	209 (83.6)
	Positive	41 (16.4)
Site 3	Negative	171 (68.4)
	Positive	79 (31.6)
Site 4	Negative	194 (77.6)
	Positive	56 (22.4)
Distribution of routine microscopy results (positive/negative) by site		

Table 4
Parasite density in routine positive slides

Site	Positive slides (n)	Mean density (parasites/ μ L)	SD	Min	Max
Site 3	81	9,124	23,960	19	137,142
Site 2	40	21,340	82,195	66	496,843
Site 4	48	19,778	46,609	12	250,000
Site 1	6	260	271	40	690
Total	175	12,626	38,259	34	221,169
Distribution of routine microscopy results (positive) and parasite density parameters for positive slides according to health facility					

Daily workload exceeded 10 slides for 27 providers (93.1%) (Table 2). Per-slide reading time was < 10 min (14/29, 48.3%) or $\geq$ 10 min (15/29, 51.7%) (Table 2). Negative declarations followed > 100 fields in 16/29 cases (55.2%) (Table 2). Only 9 providers (31.0%) routinely identified species; all quantified thick smear density but none thin films.

3.3. Expert Re-reading

Re-readings classified 885 slides (88.5%) negative and 115 (11.5%) positive (Table 5). Positivity by site: Site 4 (40/250, 16.0%), Site 3 (36/250, 14.4%), Site 2 (33/250, 13.2%), Site 1 (6/250, 2.4%). Species was identifiable in 104/115 positives (90.4%), exclusively *P. falciparum* (Table 6). Densities averaged 24,999/ μ L (Control 1; SD 51,409), 25,119/ μ L (Control 2; SD 57,109), 25,888/ μ L (Control 3; SD 54,990); range 342–408,000/ μ L (Table 7).

Table 5
Expert re-reading results by site

Site	Expert result	n (%)
Site 1	Positive	6 (2.4)
	Negative	244 (97.6)
Site 2	Positive	33 (13.2)
	Negative	217 (86.8)
Site 3	Positive	36 (14.4)
	Negative	214 (85.6)
Site 4	Positive	40 (16.0)
	Negative	210 (84.0)
Distribution of expert re-reading results (positive/negative) and proportion of slides with species identified by study site		

Table 6
Species identification among expert-positive slides

Site	Species identified	n (%) of positives
Site 1	Yes	4 (3.48)
	No	2 (1.74)
Site 2	Yes	32 (27.83)
	No	1 (0.87)
Site 3	Yes	33 (28.70)
	No	3 (2.61)
Site 4	Yes	35 (30.43)
	No	5 (4.35)

Table 7
Parasite density of control slides

Variable	Observations (n)	Mean parasites/ μ L	SD	Min	Max
Control density 1	115	24,999	51,409	505	328,727
Control density 2	115	25,119	57,109	380	408,000
Control density 3	115	25,888	54,990	342	353,000
Summary statistics of parasite density for three control readings performed by the expert readers					

3.4. Diagnostic Performance

Routine vs. reference (expert majority) yielded 69 discrepancies (6.9%; $p < 0.001$). Overall concordance reached 88.1%. Sensitivity was 78.3%, specificity 89.4%, positive predictive value 48.9%, negative predictive value 96.9% (Table 8).

Table 8
Diagnostic performance of routine microscopy compared with expert reference reading

Indicator	Estimate (%)
Sensitivity	78.3
Specificity	89.4
Positive predictive value	48.9
Negative predictive value	96.9
Sensitivity, specificity, and predictive values of routine malaria microscopy using expert majority reading as the reference standard.	

3.5. Factors Associated with Accuracy

Seniority (≥ 10 vs. <10 years: OR = 1.10, 95% CI 0.75–1.62; $p = 0.62$) and SOP availability (OR = 1.00, 95% CI 0.60–1.68; $p = 0.98$) showed no significant links to accuracy (Table 9). Lack of refresher training (OR = 2.72, 95% CI 1.81–4.06; $p < 0.001$) and reading time < 10 min (OR = 1.72, 95% CI 1.16–2.55; $p = 0.006$) associated significantly with errors.

Table 9
Factors associated with inaccurate routine microscopy readings

Factor	Category	Inaccurate n (%)	Accurate n (%)	Total n (%)	OR (95% CI)	p value
Seniority	< 10 years	50 (41.7)	346 (39.3)	396 (39.6)	1.10 (0.75–1.62)	0.62
	≥ 10 years	70 (58.3)	534 (60.7)	604 (60.4)	Reference	
Availability of SOPs	No	98 (83.1)	732 (83.0)	830 (83.0)	1.00 (0.60–1.68)	0.98
	Yes	20 (16.9)	150 (17.0)	170 (17.0)	Reference	
Refresher training	No	79 (66.4)	371 (42.1)	450 (45.0)	2.72 (1.81–4.06)	< 0.001
	Yes	40 (33.6)	510 (57.9)	550 (55.0)	Reference	
Reading time per slide	< 10 minutes	72 (61.0)	420 (47.6)	492 (50.8)	1.72 (1.16–2.55)	0.006
	≥ 10 minutes	46 (39.0)	462 (52.4)	508 (49.2)	Reference	
Association between provider and facility characteristics and the risk of inaccurate routine readings, expressed as odds ratios (ORs) with 95% confidence intervals from univariable analysis						

4. Discussion

This study illuminates persistent diagnostic challenges in Ouagadougou’s urban malaria microscopy, where routine readings overestimated positivity (18.4% vs. 11.5% expert; 6.9% discrepancies) amid moderate sensitivity (78.3%) but critically low positive predictive value (48.9%), signaling widespread false positives. Biomedical technicians dominated (79.3%), with most (> 50%) boasting > 10 years’ experience, yet 58.6% lacked refresher training a key correlate of errors (OR = 2.72, 95% CI [1.81–4.06], $p < 0.001$) as were rushed reads (< 10 min; OR = 1.72, 95% CI [1.16–2.55], $p = 0.006$). Thick smears prevailed (82.4%), *P. falciparum* exclusively identified cases, and expert densities (~ 25,000/μL) exceeded routine values, exposing low-parasitemia detection failures.

4.1. Comparison with Prior Studies

Our metrics resonate with sub-Saharan trends yet reveal urban-urban disparities. Mutabazi *et al.* (2021) [10] in Uganda documented comparable overcalling (22.7% routine vs. 15% expert) but higher sensitivity (95.8%), linking shortfalls to < 5 years’ experience paralleling our training void [10]. Ethiopian public hospitals [11] reported 78–91% concordance akin to ours, with training boosting accuracy 24-fold (AOR

= 24.3), while speciation lagged (24.7%) as in our routine 34.8% versus expert 90.4% [12, 13]. Tanzanian facilities achieved 87% detection but persistent false negatives, mirroring our robust NPV (96.9%) against PPV frailty [14]. Nationally, Burkina Faso's 2017 PNLP audit hit 66% errors our 6.9% suggests gains, though suboptimal per WHO EQA benchmarks (> 95% post-training) [2].

Recent EQA underscores context: DRC labs (2021) showed > 66% density errors from poor Giemsa, training deficits (only 33% trained), and urban overload, echoing our workload (> 93% >10 slides/day) [15]. Senegal's 2024 audit affirmed reference-lab excellence (WHO/ISO 15189) via sustained refreshers, contrasting our gaps [16]. Post-COVID analyses (2022–2025) note training erosion, with EQuAfrica Phase 2 emphasizing EQA scalability our findings advocate similar regional rollouts [17]. Seasonality (low July–November transmission) inflated our false positives, as Ouagadougou's variable parasitemia mirrors Amazon Basin declines where microscopy sensitivity drops below 10% prevalence [18].

4.2. Biological Underpinnings

P. falciparum's ring-stage subtlety at < 500/ML prone to artifact confusion underpins misses, exacerbated by thick smears' field-clogging or suboptimal Giemsa (pH-sensitive chromatin staining) [19]. Urban heterogeneity amplifies this: brief reads skimp WHO's 100 + negative fields, while workload fatigues trophozoite/gametocyte discernment [2]. Our elevated expert densities signal routine underquantification, risking resistance via ACT overtreatment.

4.3. Practical Implications

Prioritizing PNLP refreshers ($\geq$ 10-min protocols, SOP drills) could replicate Uganda's 2–4x gains [20, 21]. Urban labs demand EQA circuits and hybrid RDT-microscopy (RDT triage, microscopy confirmation), curbing waste from low PPV. Policy should mandate speciation/quantification, aligning with 2025 elimination targets amid Burkina Faso's 32% case drop [5].

4.4. Study Limitations

Site refusals and slide non-retention biased toward preserved samples, potentially understating defects. Audit awareness may have spurred better self-reporting, though blinded re-reading countered performance bias. Single-season scope limits transmission variability; no clinical correlates obscure impact. Cross-sectional design precludes causality on training effects.

4.5. Research Perspectives

Longitudinal EQA tracking post-refresher retention, benchmarking against qPCR for ultralow densities, merits pursuit [19]. Hybrid workflow cost-effectiveness, *P. falciparum* hrp2/3 genomics (RDT threats), and AI-augmented reading trials could transform urban diagnostics [17]. Multicenter audits linking errors to outcomes will guide PNLP scaling toward 2030 goals [1].

5. Conclusion

This study reveals significant quality gaps in urban malaria microscopy in Ouagadougou, with routine readings overestimating positivity (18.4% vs. 11.5% expert re-reading) and exhibiting low positive predictive value (48.9%), primarily due to absent refresher training (OR = 2.72) and rushed slide examinations. Despite experienced staff (79.3% biomedical technicians, > 50% with > 10 years), 6.9% discrepancies underscore detection failures at low *P. falciparum* parasitemia, confirming microscopy's vulnerability in high-workload urban settings.

These findings contribute novel urban-specific data to Burkina Faso's malaria diagnostics literature, bridging gaps between national PNLP audits (66% errors, 2017) and WHO EQA benchmarks, while validating training as the pivotal modifiable factor [1].

We recommend mandatory PNLP refresher courses emphasizing ≥ 10 -minute reads, SOP enforcement, and routine EQA rechecking to boost sensitivity beyond 78% and PPV toward 60%, alongside RDT-microscopy hybridization to curb overtreatment.

Future multicenter trials should validate molecular (qPCR) gold standards against expert microscopy and assess post-training retention, informing scalable interventions for Burkina Faso's 2030 elimination trajectory.

Declarations

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval [9] for this study was obtained from the Ministry of Health, Burkina Faso (approval number: [2021-040-MS/RCEN/DRSC]). Administrative authorization was obtained from the Regional Health Directorate and the participating health facilities in Ouagadougou. Written informed consent was obtained from all participating laboratory providers

Competing interests:

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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Author Contribution

P.M.S., J.A.T.K., M.C., H.T. and K.T.G. contributed to the conceptualization of the study. P.M.S., H.T., K.T.G. and J.A.T.K. contributed to the methodology. P.C.C., P.M.S., H.T., K.T.G., M.C. and senior biomedical technicians from the parasitology department of Yalgado Ouédraogo University Hospital contributed to validation. J.A.T.K., P.M.S., H.T., K.T.G. and M.C. contributed to the formal analysis. J.A.T.K., P.M.S., H.T., K.T.G., M.C. and P.C.C. contributed to the investigation. J.A.T.K., P.M.S., H.T. and K.T.G. curated the data. J.A.T.K. prepared the original draft. H.T., K.T.G., M.C., P.M.S., P.C.C. and A.Z. reviewed and edited the manuscript. All authors read and approved the final manuscript

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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