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Evaluation and Clinical Correlation of 18SrRNA and Microscopy in the Detection of *Plasmodium falciparum* among Children in Ilorin, Nigeria

[1]Sunday Ojo Joseph, [2]Abdulsalam Olalekan Funsho, [3]Sola Oyewole, [4]Ajibaye Olusola

[1,2] Parasitology unit, Zoology department, Kwara state University, Malete. Kwara state, Nigeria

[3]Department of Public Health, Birmingham City University, Birmingham, United Kingdom

[4]Malaria research laboratory, Biochemistry division, Nigerian Institute of Medical Research (NIMR), Yaba, Nigeria

Abstract: Accurate and timely diagnosis remains essential for effective malaria control, especially among children in endemic regions. While light microscopy remains the standard diagnostic tool in many healthcare settings, molecular techniques such as 18SrRNA polymerase chain reaction (PCR) offer improved sensitivity, particularly for detecting low-density or sub-microscopic infections. This study compared the diagnostic performance of 18SrRNA PCR and light microscopy in detecting Plasmodium falciparum among children aged 1–12 years in Ilorin, Nigeria, during antimalarial drug efficacy assessment. A cross-sectional diagnostic evaluation was conducted among 51 children presenting with clinical symptoms of malaria and high parasitemia density of ≥2000µl. Blood samples were collected and tested for *P. falciparum* on Days 0, 1, 2, and 3 using both microscopy and 18SrRNA PCR. Infection trends were analyzed over time, and the results were descriptively compared. Socio-demographic and environmental factors were also examined to assess potential associations with infection status. At baseline (Day 0), 100% of the children were positive by microscopy, while 96.1% tested positive by 18SrRNA PCR. PCR detected additional infections on Days 1 and 2 that microscopy missed, highlighting its greater sensitivity during parasite clearance. By Day 3, both methods detected infections in only 3.9% of the children. Although no statistically significant associations were observed between infection and factors such as age, water source, or toilet type, children exposed to open water and unimproved sanitation consistently had higher infection rates. Both microscopy and 18SrRNA PCR remains valuable for malaria diagnosis in endemic regions. However, the enhanced sensitivity of 18SrRNA PCR during post-treatment monitoring underscores its value in detecting low-density infections and assessing treatment efficacy. Integrating molecular diagnostics into research and targeted surveillance may improve case detection and malaria control outcomes, especially in high-risk pediatric populations.

Key words: 18SrRNA, PCR, Microscopy, Malaria Diagnosis, Plasmodium falciparum, Children, Nigeria, Molecular Diagnostics, Public Health, Parasite, Africa

I. INTRODUCTION

Malaria remains a major public health challenge which is endemic in tropical and sub-tropical regions of the world, especially in Nigeria, where environmental and weather conditions support the developmental cycle of female *Anopheles* mosquitoes, the vectors malaria parasites (World Health Organization, 2021; Sato, 2021; Okafor *et al*., 2019). In Nigeria, nearly half of the population is at risk of malaria, with children under 5 years and pregnant women were susceptible. In 2019, 35% (12 million) of the 33 million pregnant women in Africa were infected with malaria across 33 African countries (Godwin *et al*., 2023; Amede *et al*., 2022; WHO, 2021). *Plasmodium falciparum* is the predominant malaria parasite in Nigeria, and accurate timely diagnosis is critical for reducing morbidity and interrupting transmission (WHO, 2021). Traditionally, microscopy has been considered the diagnostic gold standard, offering species identification and parasite quantification. However, its effectiveness is highly dependent on technician expertise, equipment quality, and parasite density; it often fails to detect low-level or sub-microscopic infections (Ojurongbe *et al*., 2013; WHO, 2021). In Nigeria, community-based studies revealed microscopy sensitivity ranging from approximately 61% to 83%, reflecting significant limitations in field settings (Oyedeji *et al*., 2021; Ojurongbe *et al*., 2013).

By contrast, molecular diagnostics, particularly PCR assays targeting the Plasmodium 18SrRNA gene, boast much higher sensitivity, capable of detecting parasite densities as low as 0.02 parasites/µL (Oyedeji *et al*., 2021; WHO, 2021). Nigerian studies report PCR sensitivity between 67% and 97% compared to microscopy's 77%, underscoring PCR's utility in uncovering hidden infections (Osun *et al*., 2024; Oyedeji *et al*., 2021). Detecting these low-density infections is vital because individuals with sub-microscopic parasitemia can continue transmitting malaria despite appearing parasite-free under microscopy (Ita *et al*., 2019; Oyedeji *et al*., 2021). However, PCR remains largely inaccessible in most routine settings in Nigeria due to costs, technical demands, and infrastructure limitations (WHO, 2021).

This study evaluates and compares the performance of 18SrRNA PCR and microscopy for detecting P. falciparum in children in Ilorin, Nigeria. We analyze positivity rates for both methods across Days 0 through 3 post-treatment, exploring diagnostic sensitivity, treatment response, and detection discrepancies. Understanding these dynamics is a significant step toward improving surveillance, case management, and informing policy on integrating molecular tools into malaria control.

II. Materials and Methods

2.1. Study Area and Population

This study was conducted in Ilorin, the capital city of Kwara State, located in the North-Central geopolitical zone of Nigeria. Ilorin lies between latitude 8°30′N and longitude 4°33′E and serves as both an administrative and commercial center. The city comprises both urban and peri-urban settlements, with a mix of indigenous and migrant populations. According to the National Population Commission (NPC, 2006),

Kwara State has an estimated population of over 2.3 million, with Ilorin West, East, and South being the most populous local government areas.

Ilorin has a tropical savanna climate characterized by two distinct seasons: a rainy season (April to October) and a dry season (November to March). The mean annual rainfall is approximately 1,200 mm, and temperatures range between 22°C and 35°C (Adelekan, 2012). These climatic conditions create a suitable environment for seasonal transmission of *Plasmodium falciparum*, particularly during and shortly after the rainy season (Fayemiwo *et al*., 2020). Malaria transmission is mesoendemic and largely driven by Anopheles gambiae complex mosquitoes, the primary vectors in the region.

The target population consisted of children aged 1 to 12 years who presented with fever and high parasitemia density ≥2000μl were recruited at selected healthcare centers within Ilorin metropolis. This age group was chosen because children in this range are at heightened risk of malaria morbidity and often present with uncomplicated malaria (WHO, 2021). Children were recruited from both public and private healthcare facilities that provide outpatient services. The selection of health facilities was based on accessibility, laboratory infrastructure, and consent from health administrators.

Children were enrolled consecutively based on clinical suspicion of malaria, absence of recent antimalarial use, and parental consent. Those meeting eligibility criteria were followed from Day 0 to Day 3 to assess diagnostic performance and infection trends.

2.2. Study Design and Sample Size

A prospective longitudinal diagnostic comparison was conducted. A total of 51 children were enrolled using consecutive sampling based on inclusion criteria: (i) documented fever (≥37.5°C) or history of fever within the past 48 hours, (ii) no antimalarial treatment within the preceding 7 days, and (iii) written informed consent from parents or guardians.

The sample size was determined pragmatically based on budget constraints, ethical considerations, and laboratory processing capacity, while still ensuring statistical relevance. Previous studies comparing microscopy and PCR for malaria detection have reported significant diagnostic differences using similar or smaller sample sizes (Ojurongbe *et al*., 2013; Oyedeji *et al*., 2021). Thus, a sample of 51 children was deemed adequate to detect meaningful differences between diagnostic methods across multiple time points.

2.3. Data and Sample Collection

Each child was tested for *P. falciparum* infection using both microscopy and 18SrRNA PCR on Day 0 prior to treatment initiation. Follow-up blood samples were collected on Days 1, 2, and 3 post-treatment to monitor parasite clearance. Treatment followed the Nigerian national malaria treatment guidelines (typically artemether-lumefantrine).

2.4. Microscopy

Thick and thin blood smears were prepared from capillary finger-prick blood. Slides were stained using 10% Giemsa and examined under oil immersion (100x) by two independent experienced microscopists blinded to the PCR results. Parasite density was estimated by counting asexual parasites against 200 white

blood cells, assuming a standard WBC count of 8,000/µL. A third microscopist resolved any discrepancies between readings.

2.5. 18SrRNA Molecular Detection

Detection of *Plasmodium falciparum* was performed using a nested PCR approach**.** The initial (primary) PCR targeted the *Plasmodium* genus using the primers PLU5 (5'-CCTGTTGTTGCCAAACTTC-3') and PLU6 (5'-AAATCCGAACAGGTTTTGC-3'). Each 10 µL reaction mixture contained 2 µL of 5X FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia), 0.3 µM of each primer (forward and reverse), 5.4 µL of molecular-grade water, and 2 µL of template DNA.

Amplification was carried out in an Applied Biosystems 2720 Thermal Cycler (Thermo Fisher Scientific, USA) with the following cycling parameters: initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 5 minutes.

The secondary (nested) PCR was prepared similarly to the primary reaction, with the exception that 3 µL of the primary PCR product was used as the DNA template. The cycling conditions remained the same, except for an increased annealing temperature of 60°C.

Amplified products (5 µL) from the secondary PCR were electrophoresed on a 2% agarose gel stained with 0.5% ethidium bromide and visualized under a high-performance UV transilluminator. A 100 bp DNA ladder (Solis BioDyne, Tartu, Estonia) was used as a molecular marker to estimate the size of the PCR amplicons.

2.6. Data Analysis

Data were analyzed using SPSS version 25. Descriptive statistics, including frequencies and mean parasite counts, were computed. Diagnostic agreement between microscopy and 18SrRNA PCR was assessed using McNemar's test and Cohen's kappa coefficient. Positivity trends across Days 0–3 were visualized using line charts. Statistical significance was defined at $p < .05$.

2.7 Ethical Considerations

Ethical approval was obtained from the Ethical Review Committee under the Kwara State Ministry of Health Research Protocol. A formal request was submitted through the office of the Chief Medical Director (CMD), from whom permission was sought and granted. As part of the process, the researcher completed an online aptitude assessment, after which a certificate of participation was issued. The project design was subsequently reviewed and approved.

III. Results

3.1. Detection of *Plasmodium falciparum* by 18SrRNA and Microscopy

Infection detection was tracked from Day 0 to Day 3 using both microscopy and 18SrRNA PCR. As shown in Table 1 and Figure 1, microscopy consistently detected a higher number of infections compared to 18SrRNA.

Both 18SrRNA and microscopy detected *Plasmodium falciparum* infections in nearly all children at baseline (Day 0), with microscopy identifying 51 positive cases (100%) and 18SrRNA detecting 49 (96.1%). This close agreement supports the diagnostic validity of both methods at peak parasitemia. However, as treatment progressed, notable differences emerged. On Day 1, both methods detected infection in 25 children (49.0%), but by Day 2, 18SrRNA detected infections in 6 children (11.8%) compared to 4 (7.8%) by microscopy. By Day 3, both methods converged again, with 2 positive cases (3.9%) each.

These results suggest that 18SrRNA may be slightly more sensitive in detecting low-level parasitemia during the early clearance phase following treatment. The fact that it detected more positives on Day 2 could reflect lingering parasite DNA in circulation, which PCR can detect even after parasites are cleared from the bloodstream. This is consistent with prior studies where molecular methods outperformed microscopy, particularly for detecting sub-microscopic or recrudescent infections. This result is graphically represented in Figure 1.

Table 2: Infection Detection by 18SrRNA and Microscopy (Day 0-3)

Day	18SrRNA Positive	Microscopy Positive
Day 0	49 (96.1%)	51 (100.0%)
Day 1	25 (49.0%)	25 (49.0%)
Day 2	6 (11.8%)	4 (7.8%)
Day 3	2 (3.9%)	2 (3.9%)

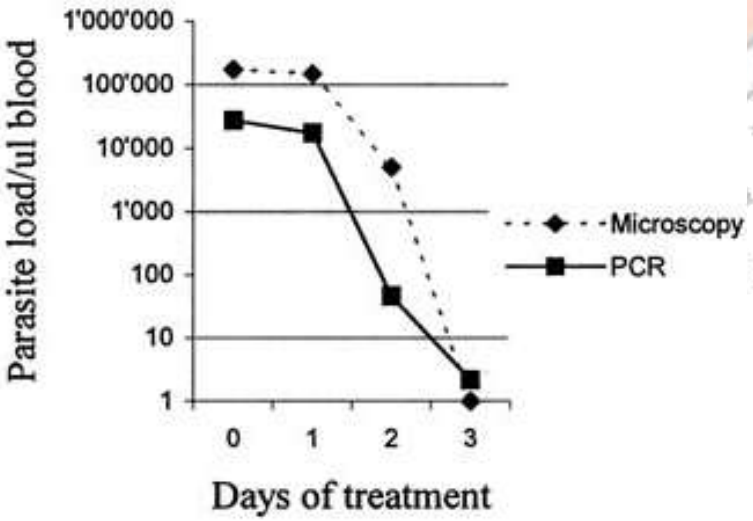


Figure 1: Trend in Infection Detection by 18SrRNA and Microscopy (Day 0–3)

3.2. Diagnostic Agreement between 18SrRNA and Microscopy (Day 0)

To assess diagnostic concordance, a cross-tabulation was performed for Day 0 and the results is indicated in Table 3. At baseline, a cross-tabulation of diagnostic outcomes revealed that all 51 children were positive by microscopy, while 49 were positive by 18SrRNA PCR. This produced a total agreement of 49 cases and two discordant cases (PCR-negative but microscopy-positive). Nonetheless, this finding still carries clinical significance. It demonstrates that PCR and microscopy results are largely consistent at peak infection, with a

slight edge in sensitivity favoring microscopy on Day 0 in this dataset. The reverse may occur during parasite clearance, where PCR retains a higher detection threshold for residual DNA, whereas microscopy cannot visualize cleared or non-viable parasites.

Table 2: Cross-tabulation of 18SrRNA and Microscopy Results (Day 0)

Method	Positive	Negative	Total
18SrRNA	49	2	51
Microscopy	51	0	51

3.3. Discussion

This study evaluated the diagnostic performance of 18SrRNA PCR compared to microscopy for detecting *Plasmodium falciparum* infection among symptomatic children in Ilorin, Nigeria. The results confirmed a high burden of malaria in the study population, with 96.1% of participants testing positive by 18SrRNA and 100% by microscopy on Day 0. These findings reinforce the hyper-endemic nature of malaria in parts of North-Central Nigeria (Fayemiwo *et al*., 2020; WHO, 2021).

Although microscopy remains the gold standard in many clinical settings, its diagnostic reliability can vary due to observer experience and low parasitemia levels (Ojurongbe *et al*., 2013). In our study, the slight discrepancy between methods on Day 0 where one child tested positive by microscopy but negative by 18SrRNA may have been due to PCR inhibition, sample quality, or parasite stage. Conversely, on Days 1 and 2, 18SrRNA detected more infections than microscopy, likely due to its higher sensitivity, especially for low-density or early-stage infections (Snounou *et al*., 1993; Oyedeji *et al*., 2021).

By Day 3, both diagnostic methods yielded the same result: only two children remained positive. This suggests that while microscopy and PCR align in capturing treatment response, PCR may provide a slightly longer window of detection during parasite clearance, since it can detect parasite DNA even when live parasites are no longer visible under a microscope. This finding mirrors results from studies in Ghana and Kenya where PCR remained positive for up to 72 hours post-treatment, while microscopy became negative earlier (Osun *et al*., 2024; Molina-de la Fuente *et al*., 2021).

The use of molecular diagnostics like 18SrRNA offers a more nuanced understanding of malaria transmission. PCR can detect submicroscopic infections, which are often missed by microscopy or RDTs, especially in partially immune or asymptomatic individuals (Snounou *et al*., 1993; WHO, 2021). In areas aiming for malaria elimination, identifying such hidden reservoirs is crucial.

IV. Conclusion

This study provides compelling evidence on the diagnostic performance of 18SrRNA-based PCR compared to microscopy in the detection of *Plasmodium falciparum* infection among children in Ilorin, Nigeria. The high prevalence of malaria observed in the sample with nearly all children testing positive on Day 0 by both methods reaffirms the burden of the disease in endemic urban settings and the urgency for

precise diagnostic approaches. While microscopy and PCR demonstrated close alignment at the onset of infection (Day 0), molecular diagnosis proved slightly more sensitive during the treatment follow-up period (Days 1 and 2), detecting low-grade infections that microscopy failed to capture. This highlights the value of PCR in identifying residual parasitemia, especially important in evaluating treatment efficacy, detecting sub-microscopic infections, and informing decisions in clinical trials or elimination programs.

The infection trends across the days indicate that both diagnostic methods are useful, but PCR offers advantages where detection at very low parasite densities is critical. The small number of cases missed by microscopy suggests that relying solely on traditional microscopy, while still valid in routine care, may underestimate parasite clearance or recrudescence, particularly in asymptomatic or partially treated cases. Although environmental and demographic variables such as age, water source, and toilet type showed meaningful patterns in relation to infection, no statistically significant associations were observed. This is likely due to the small sample size and high infection rate, which limited statistical variability. However, the observed trends align with existing literature, suggesting that exposure to unsafe water, poor sanitation, and young age remain relevant risk factors for malaria in similar settings.

In view of these findings, we recommend that molecular diagnostics like 18SrRNA PCR be incorporated into malaria research, surveillance, and case confirmation strategies, particularly in high-burden areas. While microscopy remains practical and widely accessible, especially in resource-limited settings, it should be complemented by PCR methods in situations where accurate assessment of treatment response or low-density parasitemia is required. Additionally, targeted investments in environmental health infrastructure — including improved water sources and sanitation — should accompany malaria control efforts, given the underlying social and ecological determinants that sustain transmission.

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