Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Therapeutic Efficacy of Artemether-Lumefantrine and Artesunate- Amodiaquine for the Treatment of Uncomplicated Plasmodium falciparum Malaria in South Sudan: A Multi-Site Therapeutic Efficacy Study, 2024 – 2025

Dhol Samuel Ayuen

dholsamuel@gmail.com

University of Juba

Atemthi D. Dau

University of Juba

Amanya Jacob Iboyi

Ministry of Health

Mustafa Ahmad R Lasu

World Health Organisation

Dhieu Daniel Mayen

Ministry of Health

Charles Stanley Mazinda

Ministry of Health

Apal Toby Maduot

Ministry of Health

Dhel Nhomachot Manot

Ministry of Health

Moses Deng Malual

Ministry of Health

Research Article

Keywords: Artesunate-amodiaquine (ASAQ), Artemether-lumefantrine (AL), therapeutic efficacy study (TES), Antimalarial resistance, efficacy monitoring, South Sudan

Posted Date: August 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10503406/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Malaria Journal on September 1st, 2026.
See the published version at https://doi.org/10.1186/s12936-026-06127-0.

Therapeutic Efficacy of Artemether-Lumefantrine and Artesunate-Amodiaquine for the Treatment of Uncomplicated Plasmodium falciparum Malaria in South Sudan: A Multi-Site Therapeutic Efficacy Study, 2024 – 2025

Authors: Dhol Samuel Ayuen[1 2*], Atemthi D. Dau[1 2], Amanya Jacob Iboyi[2], Mustafa Ahmad R Lasu[3], Dhieu Daniel Mayen[2], Charles Stanley Mazinda[2], Apal Toby Maduot[2], Dhel Nhomachot Manot[2], Moses Deng Malual[2]

Affiliations:

1. School of Public Health, University of Juba, Juba, South Sudan
2. National Malaria Control Programme (NMCP), Ministry of Health, Juba, South Sudan
3. WHO, South Sudan Country Office, Juba, South Sudan

***Corresponding author**

Corresponding author email address: dholsamuel@gmail.com

Abstract

(Word count: 346)

Background

In South Sudan, Artesunate-amodiaquine (ASAQ) is the first-line regimen, and Artemether-Lumefantrine (AL) is the second-line regimen for treating uncomplicated Plasmodium falciparum malaria. However, no therapeutic efficacy study (TES) has been conducted in South Sudan since the introduction of ASAQ and AL. Our TES aimed to assess the efficacy of ASAQ and AL across four sites in South Sudan.

Methods

This study was conducted in Juba, Bor, Wau, and Yambio. Children aged 6 months to 59 months with microscopically confirmed uncomplicated P. falciparum monoinfection were assigned to ASAQ (at all sites except Yambio) or AL (at Juba and Yambio) and followed for 28 days to assess treatment response. The primary endpoint was the Kaplan-Meier PCR-corrected adequate clinical and parasitological response (ACPR) on Day 28. Day 3 parasite positivity was used as a measure of early treatment response. Dried blood spots were collected to distinguish recrudescence from new infections. All enrolled participants who received treatment were included in the primary outcome analysis.

Results

Between 3rd January 2024 and 29th January 2025, 390 children were enrolled across all arms, of whom 40 (10.3%) were lost to follow-up, and 350 (89.7%) were included in the per-protocol analysis. Kaplan-Meier uncorrected ACPR was 96.5% (95% CI: 92.6 – 100.0) in Juba-AL, 95.6%

(95% CI: 90.8 – 100.0) in Juba-ASAQ, 97.7% (95% CI: 94.6 – 100.0) in Bor, 100.0% (95% CI: 100.0 – 100.0) in Wau, and 93.0% (95% CI: 87.8 – 98.6) in Yambio. Kaplan-Meier PCR-corrected ACPR exceeded 90% in all arms: Juba-AL 98.9% (95% CI: 96.6 – 100.0), Juba-ASAQ 100.0% (95% CI: 100.0 – 100.0), Bor 100.0% (95% CI: 100.0 – 100.0), Wau 100.0% (95% CI: 100.0 – 100.0), and Yambio 98.9% (95% CI: 96.8 – 100.0). Day 3 parasite positivity was notably elevated at Bor (13.8%), exceeding the WHO 10% threshold used as an indicator of suspected artemisinin partial resistance.

Conclusions

ASAQ and AL remain highly efficacious against uncomplicated P. falciparum malaria in South Sudan, supporting the continuation of the current treatment policy. The high Day 3 positivity rate at Bor warrants enhanced molecular surveillance and repeat efficacy monitoring. This study establishes a baseline for systematic national TES surveillance.

Keywords

Artesunate-amodiaquine (ASAQ), Artemether-lumefantrine (AL), therapeutic efficacy study (TES), Antimalarial resistance, efficacy monitoring, South Sudan.

BACKGROUND

Plasmodium falciparum malaria remains a leading cause of preventable morbidity and mortality worldwide, accounting for an estimated 282 million clinical cases and 610,000 deaths globally in 2024 (1). Sub-Saharan Africa bears a disproportionate share of this burden, and South Sudan is among the most severely affected nations on the continent. In 2024 alone, South Sudan recorded an estimated more than 3 million malaria cases and over 6,700 deaths, with some of the highest parasite prevalence estimates in Africa (1) (2). Transmission is intense and perennial across much of the country, shaped by ecological diversity and a prolonged rainy season. These factors are compounded by decades of armed conflict, mass population displacement, a severely fragile health system, and near-absent disease surveillance capacity. These conditions collectively amplify malaria risk and impede an effective public health response.

Artemisinin-based combination therapies (ACTs) are the World Health Organisation (WHO)-recommended first-line treatment for uncomplicated *P. falciparum* malaria worldwide (3). South Sudan's National Malaria Control Programme (NMCP) recommends artesunate-amodiaquine (ASAQ) as first-line therapy and artemether-lumefantrine (AL) as the alternative. These recommendations were based on two small efficacy studies: one conducted in Malakal, Upper Nile State (2003–2004), which documented high ASAQ efficacy (4), and another in Juba, Central Equatoria State (2004–2007), which confirmed AL efficacy (5). No therapeutic efficacy data have been generated in South Sudan since 2007. Over the past two decades, the country's epidemiological landscape has shifted dramatically, yet treatment policy has remained unchanged and is not supported by contemporary local evidence. Notably, while ASAQ is the officially recommended first-line treatment, AL is more widely dispensed in the private sector, underscoring the importance of monitoring both drugs.

Critically, artemisinin partial resistance (ART-R) has now emerged independently across Eastern and Southern Africa. Validated *kelch13* mutations have been documented at clinically significant frequencies in multiple countries: R561H in Rwanda, the earliest African focus, with evidence of de novo clonal expansion since at least 2014 (6) (7) and subsequently in Uganda (8) (9) and northwestern Tanzania (10) (11), with prevalence exceeding 40–50% in some northern districts (8) (9); R622I in Eritrea (12) and Sudan (13), and increasingly in Ethiopia (14); and P441L in Uganda and parts of Southern Africa (15). The geographic trajectory of resistance in Africa closely mirrors the pattern observed in Southeast Asia approximately 10 –15 years earlier, with resistance spreading from initial foci across borders and eventually involving entire subregions. Particularly concerning is that South Sudan shares borders with Uganda, Ethiopia, and Sudan, three countries with confirmed or emerging ART-R, placing it at direct risk of cross-border transmission of resistant parasite lineages.

Despite this rapidly evolving regional resistance landscape, no systematic, multi-site therapeutic efficacy study (TES) has ever been conducted in South Sudan. Recent TES findings from neighbouring countries in East Africa and the Horn of Africa indicate that ASAQ and AL generally remain efficacious, with PCR-corrected adequate clinical and parasitological response (ACPR) rates largely above the WHO 90% treatment-switch threshold (16) (17) (18) (19). However, AL efficacy has declined below this threshold at some sites (17) (20) (21) (22), and elevations in Day 3 parasite positivity, a marker of slowing parasite clearance and a sentinel for ART-R, have been reported in multiple study settings. The WHO recommends that national malaria control programmes conduct TES at sentinel sites at least every two years to detect early shifts in ACT efficacy and inform timely, evidence-based policy decisions (3). The complete absence of such data in South Sudan represents a critical gap: in a country with one of the continent's highest malaria burdens, surrounded by neighbours with documented resistance emergence, the NMCP has been unable to verify whether its first-line treatments remain effective or to detect early warning signals of resistance.

To address this evidence gap, we conducted the first multi-site TES in South Sudan in 2024–2025 at four geographically and epidemiologically distinct sentinel sites (Juba, Bor, Wau, and Yambio). We evaluated the efficacy and safety of AL and ASAQ in children aged 6–59 months with uncomplicated P. falciparum malaria. Molecular surveillance for Pfkelch13 mutations and partner drug resistance markers was integrated with clinical endpoints. The primary objective was to determine whether the PCR-corrected adequate clinical and parasitological response (ACPR) at Day 28 met the WHO 90% efficacy threshold for each drug at each site. Secondary objectives were to assess Day 3 parasite and fever positivity rates as indicators of early clinical and parasitological response. These data are intended to inform NMCP treatment policy directly and to establish a baseline for ongoing resistance surveillance in South Sudan.

METHODS

Study Design and Setting

We conducted a prospective, one-arm, open-label in vivo TES in accordance with the standard WHO protocol for monitoring antimalarial drug efficacy. Five study arms were established at four sites: Juba (two arms; AL and ASAQ), Bor (ASAQ), Wau (ASAQ), and Yambio (AL). The study was conducted at Nyakuron Primary Health Care Centre (PHCC) in Juba, Bor State Hospital in Bor, Sika Hadid PHCC in Wau, and Yambio PHCC in Yambio. Sites were selected to represent varying transmission intensity settings in South Sudan, based on the malaria indicator survey (MIS) (**Table 1**, **Figure 1**). Juba, the capital, is in Central Equatoria State and has high year-round transmission driven by the River Nile floodplain. Bor (Jonglei State) and Wau (Western Bahr el

Ghazal) are inland sites with intense seasonal transmission linked to flooding and agricultural practices. Yambio (Western Equatoria) lies in a high-rainfall forest belt with perennial, high-intensity transmission. All four locations have government-run health facilities and functioning malaria control programmes.

Participants

Eligible participants were children aged 6–59 months presenting to study health facilities with uncomplicated P. falciparum malaria. Inclusion criteria were: (i) age 6–59 months; (ii) mono-infection with P. falciparum, confirmed by Giemsa-stained thick blood smear, with an asexual parasite density of 1,000–200,000 parasites/μL; (iii) axillary temperature ≥37.5°C or a history of fever in the preceding 24 hours; (iv) ability to take oral medication; (v) informed consent from a parent or guardian; and (vi) willingness to attend all follow-up visits.

Exclusion criteria included: (i) signs of severe or complicated malaria according to WHO definitions; (ii) mixed Plasmodium species infection; (iii) severe malnutrition (weight-for-height z-score <−3 or bilateral pitting oedema); (iv) haemoglobin <8 g/dL; (v) known hypersensitivity to the study drugs; (vi) a history of antimalarial treatment within the preceding two weeks; and (vii) any other febrile illness that could confound assessment of treatment response.

Treatment

At Juba and Yambio, eligible participants received artemether-lumefantrine (Coartem®, Novartis) as a fixed-dose combination, with weight-based dosing according to the manufacturer's guidelines over three days (Days 0 – 2). Six doses were administered at 0, 8, 24, 36, 48, and 60 hours. Patients were encouraged to take the medication with food or a fatty drink to optimise lumefantrine absorption. At Bor, Wau, and Juba (second arm), participants received artesunate-amodiaquine (ASAQ Winthrop®, Sanofi) with weight-based dosing over three days. All doses were directly observed by trained study staff, and any vomiting or adverse reactions were recorded. Rescue treatment with artesunate injection was provided in cases of therapeutic failure or intolerance to the study drugs.

Follow-up Procedures and Outcome Classification

Participants were followed up on Days 1, 2, 3, 7, 14, 21, and 28 in accordance with the WHO protocol. At each visit, a standardised assessment was performed, including: axillary temperature measurement; preparation and reading of thick and thin blood smears; symptom assessment and adverse event monitoring at scheduled time points. Blood samples (dried blood spots (DBS)) were collected on Whatman 3MM filter paper at enrolment (Day 0) and on any day with detected parasitaemia for later PCR genotyping to distinguish recrudescence from reinfection, as well as for determination of mutations in the Pf kelch13 and of Pfhrp2 and Pfhrp3 deletions (to be reported later, as samples are still being processed).

Clinical and parasitological outcomes on Day 28 were classified according to WHO definitions into: (i) early treatment failure (ETF), defined as danger signs, severe malaria, or failure to clear parasitaemia in the presence of fever by Day 3, or a higher parasite density on Day 2 than on Day 0; (ii) late clinical failure (LCF), defined as danger signs, severe malaria, or fever with parasitaemia after Day 3 and before Day 28, without meeting ETF criteria; (iii) late parasitological failure (LPF), defined as parasitaemia on Day 7 or later in the absence of fever, without meeting ETF or LCF criteria; and (iv) adequate clinical and parasitological response (ACPR), defined as the absence of parasitaemia on Day 28, irrespective of fever, without meeting any failure criterion.

Day 3 Parasitaemia Assessment

Day 3 parasitaemia positivity was defined as the presence of asexual parasites on a Giemsa-stained thick blood smear on Day 3. A Day 3 positivity rate of ≥10% was used as an alert threshold indicative of possible reduced artemisinin susceptibility, consistent with WHO guidance. Parasite positivity rates were calculated on Days 1, 2 and 3.

Laboratory Methods

Thick and thin blood smears were prepared and stained with 10% Giemsa for 10 minutes. Parasite density was estimated by counting parasites against 200 leucocytes (or 500 if <10 parasites/200 leucocytes), assuming a standard leucocyte count of 6,000/µL. Slides were read independently by two trained microscopists, and discrepancies >50% were resolved by a third reader. Quality assurance was conducted by a WHO-accredited reference laboratory.

Polymerase chain reaction (PCR) genotyping was performed to distinguish recrudescence from reinfection in participants with recurrent parasitaemia. Day 0 and Day of failure samples were genotyped at 3 loci (pfmsp1, pfmsp2, and a microsatellite marker) following the WHO-recommended TES protocol (3). Fragments of the *pfmsp1* and *pfmsp2* genes were amplified by nested conventional PCR. Fragment sizes were determined by capillary electrophoresis on an Agilent D1000 ScreenTape Station.

For the two loci, recrudescence was Day0 and Day of failure ≤ 10 bp; reinfection was Day0 and Day of failure > 10 bp. The 3rd locus is a neutral microsatellite marker, PolyA. The marker was amplified using a fluorescently labelled primer plus an unlabeled primer (nested PCR: 30 cycles + 30 cycles), and size was determined by capillary sequencing on an ABI sequencer using GeneScan software.

For polyA, recrudescence was Day0 and Day of failure < 5 bp, whereas reinfection was Day0 and Day of failure ≥ 5 bp.

Genomic DNA was extracted from Day 0 DBS samples and from DBS samples on Day of failure using commercially available DNA extraction kits (QIAamp DNA Mini Kits, QIAGEN) and robots (QIAcube Connect, QIAGEN) following the manufacturer's instructions. DNA was eluted in a volume of 100 μL with nuclease-free water at an accredited reference laboratory. Cases were classified as reinfection if at least one new allele was detected and all Day 0 alleles were absent at the comparator locus.

Data Management and Statistical Analysis

Data were recorded on standardised case report forms and entered into a WHO TES database. The primary efficacy endpoint was PCR-corrected ACPR on Day 28, estimated using the Kaplan-Meier survival analysis method, which accounts for participants lost to follow-up or withdrawn. Participants who were lost to follow-up or withdrew were censored at the last contact date. A per-protocol analysis was performed, excluding participants who violated the protocol or were lost to follow-up.

In sample size calculations, the treatment failure rate for artemether-lumefantrine or artesunate-amodiaquine in the area is assumed to be 5%. At a 95% confidence level and 5% precision, a minimum of 73 patients must be included. Accounting for a 20% increase to allow for loss to follow-up and withdrawals during the 28-day follow-up period, 88 patients for artesunate-amodiaquine and 88 for artemether-lumefantrine were planned to be included at each site. Around

704 eligible patients were to be recruited from all 4 facilities; however, only 390 participants were recruited, owing to AL being available only in Juba and Yambio, and to the ASAQ number of participants not being achieved in Wau, when the study was stopped due to low numbers of malaria cases.

Baseline characteristics were summarised as proportions for categorical variables and as medians with interquartile range (IQR) for continuous variables. Treatment outcomes (primary and secondary) were analysed in accordance with the WHO protocol and presented by site. Loss to follow-up, withdrawal, protocol violations, and individuals with unresolved PCR results were reported as non-determined and excluded from the PCR-corrected treatment outcome analysis. Individuals with reinfection, loss to follow-up, and withdrawal were censored in the Kaplan–Meier analysis. 95% confidence intervals (CIs) in the per-protocol analysis were calculated using the Wilson score method, and in the Kaplan-Meier analysis using the Greenwood formula. All analyses were performed using R Version 4.5.2.

Ethical Considerations

The study was approved by the South Sudan Ministry of Health Research Ethics and Review Board (Reference:MOH/RERB/P/64/07/08/2023-MOH/RERB/A/64/11/10/2023). Informed consent was obtained from the parent or legal guardian of each participant before enrolment. Patient confidentiality was maintained throughout the study, and all participants received free care for malaria-related illnesses during the study period. Participants with treatment failure were treated with artesunate injection per national guidelines.

RESULTS

Enrolment and Participant Flow

Between 3rd January 2024 and 29th January 2025, 403 children were screened across all study sites, of whom 390 (96.8%) were enrolled. All 13 (3.2%) excluded children had microscopically negative results for parasites. A total of 350 participants were included in the per-protocol analysis (Juba-AL: 78, Juba-ASAQ: 66, Bor-ASAQ: 87, Wau-ASAQ: 34, Yambio-AL: 85). The reason for exclusion from the per-protocol analysis was loss to follow-up (n = 40). Participant flow is detailed in **Figure 2**. Enrolment by site and study period is summarised in **Table 1**.

Baseline Characteristics

Baseline demographic and clinical characteristics were broadly similar across study arms (**Table 2**). The median age ranged from 2 years (IQR [1.0 – 3.0]) in Yambio to 3 years (IQR [2.0 – 4.0]) in Juba. Overall, 51% of participants were male (ranging from 46.0% to 58.3% across sites). The median baseline temperature was 37°C (IQR [37.0 – 37.8]). The median parasite density at enrolment was 17,590 parasites/μL (IQR [5,896 – 59,503]), with the highest median density observed in Yambio (92,712 p/μL; IQR [17,770 – 177,920]), followed by Bor (27,665 p/μL; IQR [9,496 – 73,749]). Gametocytes were detected at enrolment in 1.5% of participants overall.

Treatment Outcomes and PCR-Corrected Efficacy

The primary outcomes are summarised in **Table 3**. Crude (PCR-uncorrected) ACPR was high across all sites, indicating fewer recurrences. Kaplan-Meier Day 28 PCR-uncorrected ACPR estimates were: Juba-AL 96.5% (95% CI [92.6 – 100.0]), Juba-ASAQ 95.6% (95% CI [90.8 – 100.0]), Bor 97.7% (95% CI [94.6 – 100.0]), Wau 100% (95% CI [100.0 – 100.0]), and Yambio 93.0% (95% CI [87.8 – 98.6]). Overall, 14 recurrences were detected and classified by PCR: 9 were reinfections (new infections), 2 were recrudescences (true treatment failures), and 3 were inconclusive. All confirmed recrudescences were early treatment failures and occurred in the AL treatment arms.

PCR-corrected Kaplan-Meier ACPR at Day 28 exceeded the WHO 90% efficacy threshold across all study arms. Kaplan-Meier estimates of PCR-corrected ACPR were: Juba-AL 98.9% (95% CI [96.6 –100.0]), Juba-ASAQ 100% (95% CI [100.0 – 100.0]), Bor 100% (95% CI [100.0 – 100.0]), Wau 100% (95% CI [100.0 – 100.0]), and Yambio 98.9% (95% CI [96.8 – 100.0]). The per-protocol analysis also showed Day 28 efficacy estimates above 90%, although the lower bound of the confidence interval in Wau was below 90% (**Table 3).** The Kaplan-Meier curves are shown in **Figure 3.**

Secondary outcomes

The secondary outcomes were the prevalence of fever and the parasite positivity rate on Days 1, 2, and 3 after treatment initiation. Day 3 parasite positivity rates varied substantially across study arms, ranging from 0% in Yambio to 13.8% in Bor (**Table 4**). By contrast, Day 3 fever prevalence

ranged from 0% in Bor to 7.5% in Yambio. Bor was the only site where the Day 3 positivity rate exceeded the WHO alert threshold of 10% for artemisinin partial resistance.

None of the participants who were Day 3 positive in Bor subsequently experienced treatment failure (LCF or LPF). All recurrences in Bor were new infections rather than treatment failures due to reduced drug susceptibility.

DISCUSSION

This study presents the first multi-site assessment of the therapeutic efficacy of artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) in South Sudan. Both regimens met the WHO ≥90% PCR-corrected ACPR threshold at all four sites on Day 28, supporting the continued use of the current national first- and second-line treatment policy. Nonetheless, two findings warrant attention: the wide confidence interval for ASAQ in Wau, with the lower bound falling below 90% in the per-protocol analysis, and, most critically, a Day 3 parasite positivity rate of 13.8% in Bor, exceeding the WHO 10% alert threshold for suspected artemisinin partial resistance (ART-R) (3) (23).

The high PCR-corrected ACPR estimates observed here are consistent with recent TES data from East Africa and the Horn of Africa, where both AL and ASAQ have generally retained efficacy above 90% (11) (16) (18) (22). Nonetheless, several regional studies have documented AL efficacy below the WHO policy-change threshold of 90%. Ebong et al. in Uganda reported that PCR-corrected AL efficacy at Busia and Arua had 95% confidence intervals that fell below 90% (20), a finding particularly relevant given Arua's geographical proximity to South Sudan's border and well-documented cross-border population movement. More recently, Kamya et al. confirmed declining AL efficacy at multiple Ugandan sites in a four-arm randomised trial (17), and similar signals have been reported in Kenya (22) and the Democratic Republic of Congo (DRC) (21). Against this regional backdrop, the high efficacy documented at all four South Sudan sites is reassuring. However, it is important to recognise that high clinical ACPR does not exclude the presence of early ART-R: residual parasites not cleared by the artemisinin component may still be rescued by an efficacious partner drug, masking underlying resistance at the level of clinical outcome (24). This interpretation is consistent with the dissociation between Day 3 positivity and treatment failure observed at Bor in this study. Comprehensive molecular surveillance of *Pfkelch13*, *Pfmdr1*, and *Pfcrt,* paired with future TES, is therefore essential to distinguish between these scenarios.

The ASAQ per-protocol confidence interval at Wau, whose lower bound fell marginally below 90%, should be interpreted cautiously. This reflects imprecision arising from a small effective sample size, a recognised challenge in resource-constrained, low-enrolment settings, rather than a

signal of a true decline in efficacy. A repeat TES with adequate power at Wau is warranted to confirm ASAQ efficacy at that site.

The most clinically significant finding of this study is the 13.8% Day 3 parasite positivity rate in Bor, which exceeds WHO's 10% alert threshold for suspected ART-R. Day 3 positivity is an established pharmacodynamic marker of artemisinin susceptibility: the artemisinin component of ACTs acts primarily on ring-stage parasites, rapidly reducing peripheral parasitaemia within the first 48–72 hours (25) (26). When >10% of patients remain slide-positive on Day 3, this signals slowed parasite clearance and prompts WHO recommendations for enhanced surveillance and formal clearance-rate assessment (3). Importantly, the 10% threshold was derived from studies in high-transmission settings in Africa, and mathematical modelling has shown that its predictive value is sensitive to baseline parasitaemia and sample size (27). Therefore, the threshold should be interpreted alongside these parameters rather than in isolation.

The elevated Day 3 positivity at Bor was not accompanied by a corresponding excess of PCR-corrected treatment failures, and several non-resistance explanations must be considered. First, higher baseline parasite densities at Bor could independently prolong clearance time even in a fully artemisinin-sensitive parasite population. Second, imperfect drug absorption may have led to subtherapeutic drug exposure. Third, pharmacokinetic variability in amodiaquine metaboliser status could reduce effective drug concentrations in a subset of participants. Fourth, and most concerningly, the Bor signal may represent the early, pre-failure stages of emerging ART-R, in which the partner drug's residual efficacy still compensates for slowed artemisinin-mediated clearance, mirroring the pattern observed in northern Uganda before widespread molecular confirmation of *Pfkelch13* A675V and C469Y mutations (8) (9).

Pending *Pfkelch13* genotyping results from samples collected in Bor, which are still being processed and will be reported separately, a definitive mechanistic attribution is not yet possible. Confirmation of ART-R at Bor would require a prevalence of ≥5% of a WHO-validated *Pfkelch13* mutation alongside the observed Day 3 positivity, and, ideally, a formal parasite clearance half-life assessment by serial 6-hourly sampling (25). A parasite clearance half-life ≥5 hours is the definitive in vivo phenotype of ART-R, although this may not hold in high transmission-intensity settings like South Sudan (28).

These findings underscore the urgent need for institutionalised, periodic TES surveillance across South Sudan. South Sudan borders Uganda, Ethiopia, and Sudan, three countries with confirmed or emerging *Pfkelch13*-mediated ART-R (8) (9) (13). Cross-border population movement is substantial, particularly in Bor (Jonglei State), which lies along major migration and trade routes. Bor should be prioritised for an expedited repeat TES, ideally integrated with rigorous molecular surveillance, including whole-genome sequencing, to characterise parasite population structure

and identify known and emerging resistance alleles. In the interim, the NMCP should consider enhanced clinical monitoring in Bor and prepare contingency guidance should *Pfkelch13* genotyping confirm validated resistance mutations.

The high overall efficacy documented in Juba, Wau, and Yambio provides a critical national baseline for future surveillance comparisons. Establishing a permanent sentinel TES network with standardised data collection, sample archiving for molecular work, and a defined schedule of repeat surveys at each site is now an operational priority for the NMCP and its partners. The WHO recommends TES every two years, and given the pace of ART-R expansion in the region, South Sudan cannot afford a second two-decade gap in efficacy evidence.

This study has important strengths. It is the first systematic, multi-site TES in South Sudan, covering four geographically and epidemiologically distinct sites and providing nationally representative baseline efficacy data. Strict adherence to WHO TES methodology ensures international comparability and suitability for policy use.

Several limitations should be acknowledged. First, *Pfkelch13* genotyping is incomplete; results are pending for a subset of samples from Bor, and a mechanistic interpretation of the Day 3 signal must await these data. Second, per-protocol confidence intervals for ASAQ in Wau were wide due to a small effective sample size, limiting precision at that site. Third, the study enrolled children aged 6–59 months, so findings may not generalise to adults or pregnant women, who differ in immune status and pharmacokinetics. Fourth, Day 3 parasitaemia was assessed by light microscopy; more sensitive methods, such as quantitative PCR, would detect submicroscopic parasitaemia and likely increase the observed positivity rate, meaning our Day 3 estimate at Bor may be conservative. Fifth, pharmacokinetic data were not collected, limiting interpretation of the Day 3 findings. Sixth, the 28-day follow-up window may miss late recrudescence driven by waning partner drug efficacy, which typically manifests after Day 28 for lumefantrine and after Day 42 for longer half-life drugs (29).

CONCLUSIONS

In this first multi-site TES in South Sudan, both AL and ASAQ demonstrated high therapeutic efficacy against uncomplicated *P. falciparum* malaria across all four study sites, with PCR-corrected Day 28 ACPR rates meeting the WHO ≥90% threshold. These findings provide the first contemporary evidence base supporting continuation of the current national ACT treatment policy and fill a critical two-decade data gap.

However, the 13.8% Day 3 parasite positivity rate at Bor, exceeding the WHO alert threshold for suspected ART-R, is a sentinel signal that demands an urgent and structured response. Pending *Pfkelch13* genotyping data from Bor, which will be essential to determine whether this signal is

attributable to validated artemisinin resistance alleles, Bor should be prioritised for a repeat TES and a formal parasite clearance half-life study.

More broadly, these results demonstrate both the feasibility and the indispensability of systematic TES surveillance in South Sudan. Establishing a national sentinel TES network with integrated molecular surveillance is now a public health priority. The experience in East Africa, where resistance expanded rapidly from initial clinical signals to population-level treatment failures within a few years, illustrates the cost of delayed surveillance. South Sudan must institutionalise regular efficacy monitoring to detect emerging resistance early, protect the longevity of current first-line treatments, and enable timely, evidence-based revision of treatment policy before therapeutic failure becomes entrenched.

LIST OF ABBREVIATIONS

ACTs: Artemisinin-based Combination Therapies

WHO: World Health Organisation

NMCP: National Malaria Control Programme

AL: Artemether-lumefantrine

ASAQ: Artesunate-amodiaquine

ART-R: Artemisinin partial Resistance

TES: Therapeutic Efficacy Study

ACPR: Adequate Clinical and Parasitological Response

PCR: Polymerase Chain Reaction

PHCC: Primary Health Care Centre

MIS: Malaria Indicator Survey

DBS: Dried Blood Spot

ETF: Early Treatment Failure

LCF: Late Clinical Failure

LPF: Late Parasitological Failure

IQR: Interquartile Range

CI: Confidence interval

DRC: Democratic Republic of Congo

Pfmdr1: Plasmodium falciparum multidrug resistance 1 gene

Pfcrt: Plasmodium falciparum chloroquine resistance transporter gene

DECLARATIONS

Ethical approval and consent to participate

The study was approved by the South Sudan Ministry of Health Research Ethics and Review Board (Reference:MOH/RERB/P/64/07/08/2023-MOH/RERB/A/64/11/10/2023). Informed consent was

obtained from the parent or legal guardian of each participant before enrolment. Patient confidentiality was maintained throughout the study, and all participants received free care for malaria-related illnesses during the study period. Participants with treatment failure were treated with artesunate injection per national guidelines.

Acknowledgements

The authors thank the children who participated in this study, as well as their parents and guardians. We acknowledge the Director General for Preventive Health Services at the Ministry of Health, Madam Yar Mayuon Mayen; the dedicated study teams at all four sites; national malaria control programme staff; and laboratory personnel who contributed to this work. We are grateful to the Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI) for quality assurance of blood smear microscopy and PCR genotyping. We are equally grateful to WHO-South Sudan, UNICEF, and the Global Fund to Fight AIDS, Tuberculosis and Malaria.

Funding

The conduct of this first TES was funded by the Global Fund to Fight AIDS, Tuberculosis and Malaria. The funders had no role in study design, data collection, analysis, interpretation, or the decision to submit for publication.

Competing Interests

All authors declare no competing interests.

Author Contributions

Author contributions: Conceptualisation: MDM, ADD, DNM, DSA; Methodology: DSA, ADD, DNM, AJI, MAL, CSM; Investigation: ADD, DNM, CSM, DSA; Formal analysis: DSA; Writing – original draft: DSA; Writing – review & editing: all authors; Supervision: ADD, ATM, DNM, CSM; Funding acquisition: MDM, ADD, ATM, DNM.

Data Availability

De-identified participant-level data and statistical analysis code will be made available upon reasonable request to the corresponding author, subject to approval by the South Sudan Ministry of Health and the study ethics committee.

REFERENCES

1. World malaria report 2025 [Internet]. [cited 2026 Jun 15]. Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2025

2. Weiss DJ, Dzianach PA, Saddler A, Lubinda J, Browne A, McPhail M, et al. Mapping the global prevalence, incidence, and mortality of *Plasmodium falciparum* and *Plasmodium vivax* malaria, 2000–22: a spatial and temporal modelling study. The Lancet. 2025 Mar 22;405(10483):979–90. doi:10.1016/S0140-6736(25)00038-8

3. Tools for monitoring antimalarial drug efficacy [Internet]. [cited 2025 Nov 11]. Available from: https://www.who.int/teams/global-malaria-programme/case-management/drug-efficacy-and-resistance/tools-for-monitoring-antimalarial-drug-efficacy

4. van den Broek I, Amsalu R, Balasegaram M, Hepple P, Alemu E, Hussein EB, et al. Efficacy of two artemisinin combination therapies for uncomplicated falciparum malaria in children under 5 years, Malakal, Upper Nile, Sudan. Malar J. 2005 Feb 24;4:14. doi:10.1186/1475-2875-4-14 PubMed PMID: 15730557; PubMed Central PMCID: PMC554764.

5. Elamin SB, Awad AI, Eltayeb IB, Elmardi KA, Al Hassan AH, Mohamed AO, et al. Descriptive study on the efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Sudan. Eur J Clin Pharmacol. 2010 Mar 1;66(3):231–7. doi:10.1007/s00228-009-0750-4

6. Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26(10):1602–8. doi:10.1038/s41591-020-1005-2 PubMed PMID: 32747827; PubMed Central PMCID: PMC7541349.

7. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, et al. Association of Plasmodium falciparum kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. Lancet Infect Dis. 2021 Aug;21(8):1120–8. doi:10.1016/S1473-3099(21)00142-0 PubMed PMID: 33864801; PubMed Central PMCID: PMC10202849.

8. Conrad MD, Asua V, Garg S, Giesbrecht D, Niaré K, Smith S, et al. Evolution of Partial Resistance to Artemisinins in Malaria Parasites in Uganda. N Engl J Med. 2023 Aug 23;389(8):722–32. doi:10.1056/NEJMoa2211803

9. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, et al. Evidence of Artemisinin-Resistant Malaria in Africa. N Engl J Med. 2021 Sep 22;385(13):1163–71. doi:10.1056/NEJMoa2101746

10. Juliano JJ, Giesbrecht DJ, Simkin A, Fola AA, Lyimo BM, Pereus D, et al. Prevalence of mutations associated with artemisinin partial resistance and sulfadoxine-pyrimethamine resistance in 13 regions in Tanzania in 2021: a cross-sectional survey. Lancet Microbe. 2024

Oct;5(10):100920. doi:10.1016/S2666-5247(24)00160-5 PubMed PMID: 39159629; PubMed Central PMCID: PMC11464622.

11. Ishengoma DS, Mandara CI, Bakari C, Fola AA, Madebe RA, Seth MD, et al. Evidence of artemisinin partial resistance in northwestern Tanzania: clinical and molecular markers of resistance. Lancet Infect Dis. 2024 Nov;24(11):1225–33. doi:10.1016/S1473-3099(24)00362-1 PubMed PMID: 39159633; PubMed Central PMCID: PMC11511676.

12. Mihreteab S, Platon L, Berhane A, Stokes BH, Warsame M, Campagne P, et al. Increasing Artemisinin-Resistant HRP2-Negative Malaria in Eritrea. N Engl J Med. 2023 Sep 28;389(13):1191–202. doi:10.1056/NEJMoa2210956 PubMed PMID: 37754284; PubMed Central PMCID: PMC10539021.

13. L'Episcopia M, Talha AA, Nour BYM, Sana IMA, Caspar E, Thiebaut L, et al. High Prevalence of Artemisinin-Resistant Plasmodium falciparum, Southeastern Sudan. Emerg Infect Dis. 2025 Jun;31(6):1211–5. doi:10.3201/eid3106.241810 PubMed PMID: 40439506; PubMed Central PMCID: PMC12123913.

14. Fola AA, Feleke SM, Mohammed H, Brhane BG, Hennelly CM, Assefa A, et al. Plasmodium falciparum resistant to artemisinin and diagnostics have emerged in Ethiopia. Nat Microbiol. 2023;8(10):1911–9. doi:10.1038/s41564-023-01461-4 PubMed PMID: 37640962; PubMed Central PMCID: PMC10522486.

15. Martinez-Vega R, Ishengoma DS, Gosling R. Emerging Artemisinin Partial Resistance in Southern Africa. Am J Trop Med Hyg. 2026 Mar 17;1(aop). doi:10.4269/ajtmh.25-0417

16. Laury JE, Mugittu K, Kajeguka DC, Kamugisha E, Ishengoma DS, Mandara CI, et al. Efficacy and Safety of Artemether-Lumefantrine Against Uncomplicated Falciparum Malaria Infection in Tanzania, 2022: A Single-Arm Clinical Trial. J Infect Dis. 2025 Feb 4;231(1):251–9. doi:10.1093/infdis/jiae425 PubMed PMID: 39186698.

17. Kamya MR, Nankabirwa JI, Ebong C, Asua V, Kiggundu M, Orena S, et al. Efficacies of artemether–lumefantrine, artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine for the treatment of uncomplicated *Plasmodium falciparum* malaria in children aged 6 months to 10 years in Uganda: a randomised, open-label, phase 4 clinical trial. Lancet Infect Dis. 2026 Jan 1;26(1):67–78. doi:10.1016/S1473-3099(25)00407-4

18. Daka D, Woldeyes D, Golassa L, Alemayehu GS, Zewde Z, Tamiru G, et al. Therapeutic efficacy of artemether–lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Arba Minch Zuria District, Gamo Zone, Southwest Ethiopia. Malar J. 2024 Sep 17;23(1):282. doi:10.1186/s12936-024-05087-7

19. Adam M, Nahzat S, Kakar Q, Assada M, Witkowski B, Tag Eldin Elshafie A, et al. Antimalarial drug efficacy and resistance in malaria-endemic countries in HANMAT-PIAM_net countries of the Eastern Mediterranean Region 2016–2020: Clinical and genetic studies. Trop Med Int Health. 2023;28(10):817–29. doi:10.1111/tmi.13929

20. Ebong C, Sserwanga A, Namuganga JF, Kapisi J, Mpimbaza A, Gonahasa S, et al. Efficacy and safety of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria and prevalence of molecular markers associated with artemisinin and partner drug resistance in Uganda. Malar J. 2021 Dec 24;20(1):484. doi:10.1186/s12936-021-04021-5

21. Moriarty LF, Nkoli PM, Likwela JL, Mulopo PM, Sompwe EM, Rika JM, et al. Therapeutic Efficacy of Artemisinin-Based Combination Therapies in Democratic Republic of the Congo and Investigation of Molecular Markers of Antimalarial Resistance. Am J Trop Med Hyg. 2021 Oct;105(4):1067–75. doi:10.4269/ajtmh.21-0214 PubMed PMID: 34491220; PubMed Central PMCID: PMC8592145.

22. Westercamp N, Owidhi M, Otieno K, Chebore W, Buff AM, Desai M, et al. Efficacy of Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine for the Treatment of Uncomplicated Plasmodium falciparum Malaria among Children in Western Kenya, 2016 to 2017. Antimicrob Agents Chemother. 2022 Aug 29;66(9):e00207-22. doi:10.1128/aac.00207-22

23. Das D, Price RN, Bethell D, Guerin PJ, Stepniewska K. Early parasitological response following artemisinin-containing regimens: a critical review of the literature. Malar J. 2013 Apr 19;12:125. doi:10.1186/1475-2875-12-125 PubMed PMID: 23602021; PubMed Central PMCID: PMC3649884.

24. Conrad MD, Rosenthal PJ. Antimalarial drug resistance in Africa: the calm before the storm? Lancet Infect Dis. 2019 Oct 1;19(10):e338–51. doi:10.1016/S1473-3099(19)30261-0

25. Stepniewska K, Ashley E, Lee SJ, Anstey N, Barnes KI, Binh TQ, et al. In Vivo Parasitological Measures of Artemisinin Susceptibility. J Infect Dis. 2010 Feb 15;201(4):570–9. doi:10.1086/650301 PubMed PMID: 20085495; PubMed Central PMCID: PMC4291277.

26. White N. The parasite clearance curve. Malar J. 2011 Sep 22;10:278. doi:10.1186/1475-2875-10-278 PubMed PMID: 21939506; PubMed Central PMCID: PMC3195204.

27. White LJ, Flegg JA, Phyo AP, Wiladpai-ngern JH, Bethell D, Plowe C, et al. Defining the in vivo phenotype of artemisinin-resistant falciparum malaria: a modelling approach. PLoS Med. 2015 Apr;12(4):e1001823. doi:10.1371/journal.pmed.1001823 PubMed PMID: 25919029; PubMed Central PMCID: PMC4412633.

28. White NJ, Chotivanich K. Artemisinin-resistant malaria. Clin Microbiol Rev. 37(4):e00109-24. doi:10.1128/cmr.00109-24 PubMed PMID: 39404268; PubMed Central PMCID: PMC11629630.

29. Dahal P, Simpson JA, Abdulla S, Achan J, Adam I, Agarwal A, et al. Temporal distribution of Plasmodium falciparum recrudescence following artemisinin-based combination therapy: an individual participant data meta-analysis. Malar J. 2022 Mar 24;21(1):106. doi:10.1186/s12936-021-03980-z

TABLES and FIGURES

TABLES

Table 1. Study sites, treatment arms, and participant enrolment across the five study arms in South Sudan, 2024–2025.

Study Site	Treatment Arm	State	Enrolled (n)	Per Protocol (n)	Study Period
Juba	AL	Central Equatoria	87	78	January 2024 – November 2024
Juba	ASAQ	Central Equatoria	87	66	January 2024 – May 2024
Bor	ASAQ	Jonglei	87	87	November 2024 – January 2025
Wau	ASAQ	Western Bahr el Ghazal	36	34	May 2024 – January 2025
Yambio	AL	Western Equatoria	93	85	November 2024 – December 2024
Total	—	—	**390**	**350**	—

AL = artemether-lumefantrine; ASAQ = artesunate-amodiaquine.

604 **Table 2. Baseline demographic, clinical, and parasitological characteristics by study arm.**

605

	Artesunate-Amodiaquine			Artemether-Lumefantrine	
Characteristic	**Bor N = 87**	**Juba-ASAQ N = 87**	**Wau N = 36**	**Juba-AL N = 87**	**Yambio N = 93**
Age (Years), Median (IQR)	2(2.0 – 4.0)	3(2.0 – 4.0)	3(2.0 –3.5)	3(2.0 – 4.0)	2(1.0 –3.0)
Sex, n (%)					
Female	47/87(54.0%)	40/87(46.0%)	15/36(41.7%)	43/87(49.4%)	46/93(49.5%)
Male	40/87(46.0%)	47/87(54.0%)	21/36(58.3%)	44/87(50.6%)	47/93(50.5%)
Weight (kg), Median (IQR)	12(10.0 –14.3)	13(10.0 –15.5)	11(10.0 –14.5)	13(10.0 –15.4)	10(8.0 –13.0)
Age category, n (%)					
Under 5	84/86(97.7)	87/87(100.0%)	36/36(100.0%)	86/86(100.0%)	93/93(100.0%)
5-15	2/86(2.3)	0/87(0.0%)	0/36(0.0%)	0/86(0.0%)	0/93(0.0%)
Parasite density (per µl), Median (IQR)	27,665(9,496.0 –73,749.0)	5,483(3,695.0 – 13,056.5)	18,801(7,458.0 – 49,365.0)	12,558(5,861.0 – 25,296.5)	92,712(17,770.0 –177,920.0)
Gametocyte prevalence, n (%)	0/85(0.0%)	1/87(1.1%)	0/36(0.0%)	5/87(5.7%)	0/93(0.0%)
Temperature (°C), Median (IQR)	38.0(37.0 –38.7)	37.0(37.0 – 37.4)	38(37–38.1)	37(37.0 –37.3)	38(37.0 –38.2)
Fever prevalence, n (%)	73/86(84.9%)	85/87(97.7%)	36/36(100.0%)	87/87(100.0%)	92/93(98.9%)

606 *IQR = Interquartile range*

607
608
609
610
611
612
613
614
615
616
617
618
619
620

Table 3. Clinical and parasitological treatment outcomes at Day 28 by study arm, uncorrected and PCR-corrected results, per protocol and Kaplan-Meier

	Artesunate-Amodiaquine			Artemether-Lumefantrine	
Outcome	**Bor N = 87**	**Juba-ASAQ N = 66**	**Wau N = 34**	**Juba-AL N = 78**	**Yambio N = 85**
Uncorrected outcomes, n /N (%) [95% CI]					
ACPR Day 28	85/87(97.7%) [95% CI: 91.2 – 99.6]	63/66(95.5%) [95% CI:86.4 – 98.8]	34/34(100.0%) [95% CI: 87.4 – 100.0]	75/78(96.2%) [95% CI: 88.4 –99.0]	79/85(92.9%) [95% CI: 84.7 – 97.1]
ETF	0/87(0.0%) [95% CI: 0.0 – 5.3]	0/66(0.0%) [95% CI: 0.0 – 6.85]	0/34(0.0%) [95% CI: 0.0 –12.6]	1/78(1.3%) [95% CI: 0.1– 7.9]	1/85(1.2%) [95% CI: 0.1– 7.3]
LCF	1/87(1.1%) [95% CI: 0.1– 7.1]	0/66(0.0%) [95% CI: 0.0 – 6.9]	0/34(0.0) [95% CI: 0.0 – 12.6]	0/78(0.0%) [95% CI: 0.0 – 5.9]	2/85(2.4%) [95% CI: 0.4 – 9.0]
LPF	1/87(1.1%) [95% CI: 0.1– 7.1]	3/66(4.5%) [95% CI: 1.2– 13.6]	0/34(0.0%) [95% CI: 0.0 –12.6]	2/78(2.6%) [95% CI: 0.5 – 9.8]	3/85(3.5%) [95% CI: 0.9 – 10.7]
Withdrawn / LTFU	0/87 (0.0%)	21/87 (24.1%)	2/36 (5.6%)	9/87 (10.3%)	8/93 (8.6%)
PCR-corrected outcomes, n /N (%) [95% CI]					
ACPR Day 28	85/85(100.0%) [95% CI: 94.6 – 100.0]	63/63(100.0%) [95% CI: 92.8 – 100.0]	34/34(100.0%) [95% CI:87.4– 100.0]	75/76 (98.7%) [95% CI: 91.9– 99.9]	79/80(98.8%) [95% CI: 92.3– 99.9]
ETF	0/85(0.0%) [95% CI: 0.0 – 5.4]	0/63(0.0%) [95% CI: 0.0 – 7.2]	0/34(0.0%) [95% CI: 0.0 – 12.6]	1/76(1.3%) [95% CI: 0.1– 8.1]	1/80(1.3%) [95% CI: 0.1– 7.7]
LCF	0/85(0.0%) [95% CI: 0.0 – 5.4]	0/63(0.0%) [95% CI: 0.0 – 7.2]	0/34(0.0%) [95% CI: 0.0 – 12.6]	0/76(0.0%) [95% CI: 0.0 – 6.0]	0/80(0.0%) [95% CI: 0.0 – 5.7]
LPF	0/85(0.0%) [95% CI: 0.0 – 5.4]	0/63(0.0%) [95% CI: 0.0 – 7.2]	0/34(0.0%) [95% CI: 0.0 – 12.6]	0/76(0.0%) [95% CI: 0.0 – 6.0]	0/80(0.0%) [95% CI: 0.0 – 5.7]
New infections (Re-infections)	0/85 (0.0%)	3/66 (4.5)	0/34 (0.0%)	2/78 (2.6%)	4/84 (4.8%)
Recrudescences	0/85 (0.0%)	0/63 (0.0%)	0/34 (0.0%)	1/76 (1.3%)	1/80 (1.3)
Unknown	2/87(2.3%)	0/66(0.0%)	0/34(0.0%)	0/78(0.0%)	1/85(1.2%)
Kaplan-Meier Cumulative Day 28 efficacy, (%) (95% CI)					

	Artesunate-Amodiaquine			Artemether-Lumefantrine	
Outcome	**Bor N = 87**	**Juba-ASAQ N = 66**	**Wau N = 34**	**Juba-AL N = 78**	**Yambio N = 85**
Uncorrected ACPR	97.7% (95% CI :94.6 –100.0)	95.6(95% CI: 90.8 –100.0)	100% (95% CI: 100 –100.0)	96.5% (95% CI: 92.6 – 100.0)	93.0% (95% CI: 87.8 –98.6)
PCR-corrected ACPR	100% (95% CI: 100.0 –100.0)	100% (95% CI; 100.0 –100.0)	100% (95% CI: 100.0 –100.0)	98.9% (95% CI: 96.6 – 100.0)	98.9% (95% CI: 96.8 –100.0)

624
625 *ACPR = adequate clinical and parasitological response; ETF = early treatment failure; LCF = late clinical failure;*
626 *LPF = late parasitological failure; LTFU = lost to follow-up; PCR = polymerase chain reaction. K-M estimates*
627 *account for censoring. CI = confidence interval.*

628
629

630 **Table 4. Days 1,2 and 3 parasite positivity rates and fever prevalence by study arm.**

631

	Artesunate-Amodiaquine			Artemether-Lumefantrine	
Secondary outcome	**Bor**	**Juba–ASAQ**	**Wau**	**Juba–AL**	**Yambio**
	N = 87	**N = 87**	**N = 36**	**N = 87**	**N = 93**
Day 1 parasite positivity rate, n/N (%)	68/87(78.2%)	8/85 (9.4%)	8/36(22.2%)	42/87(48.3%)	57/93(61.3%)
Day 2 parasite positivity rate, n/N (%)	27/87(31.0%)	1/83(1.2%)	0/35(0.0%)	5/87(5.7%)	4/93(4.3%)
Day 3 parasite positivity rate, n/N (%)	12/87(13.8%)*	1/81(1.2%)	1/35(2.9%)	2/87(2.3%)	0/93(0.0%)
Day 1 fever prevalence, n/N (%)	5/87(5.7%)	0/85(0.0%)	3/36(8.3%)	0/87(0.0%)	24/93(25.8%)
Day 2 fever prevalence, n/N (%)	1/87(1.1%)	0/83(0.0%)	1/35(2.9%)	0/87(0.0%)	5/93(5.4%)
Day 3 fever prevalence, n/N (%)	0/87(0.0%)	1/81(1.2%)	1/35(2.9%)	0/87(0.0%)	7/93(7.5%)

632

633 *Parasite positivity is defined as the presence of asexual P. falciparum parasites on a thick blood smear. The WHO*

634 *alert threshold for Day 3 positivity is ≥10%. IQR = interquartile range; p/µL = parasites per microlitre. *Bor*

635 *exceeded the WHO 10% alert threshold.*

636

637

FIGURES

Figure 1. Map of South Sudan showing the four study sites.

Study sites are marked by red dots: Juba (Central Equatoria, two arms: AL and ASAQ), Bor (Jonglei, ASAQ), Wau (Western Bahr el Ghazal, ASAQ), and Yambio (Western Equatoria, AL).

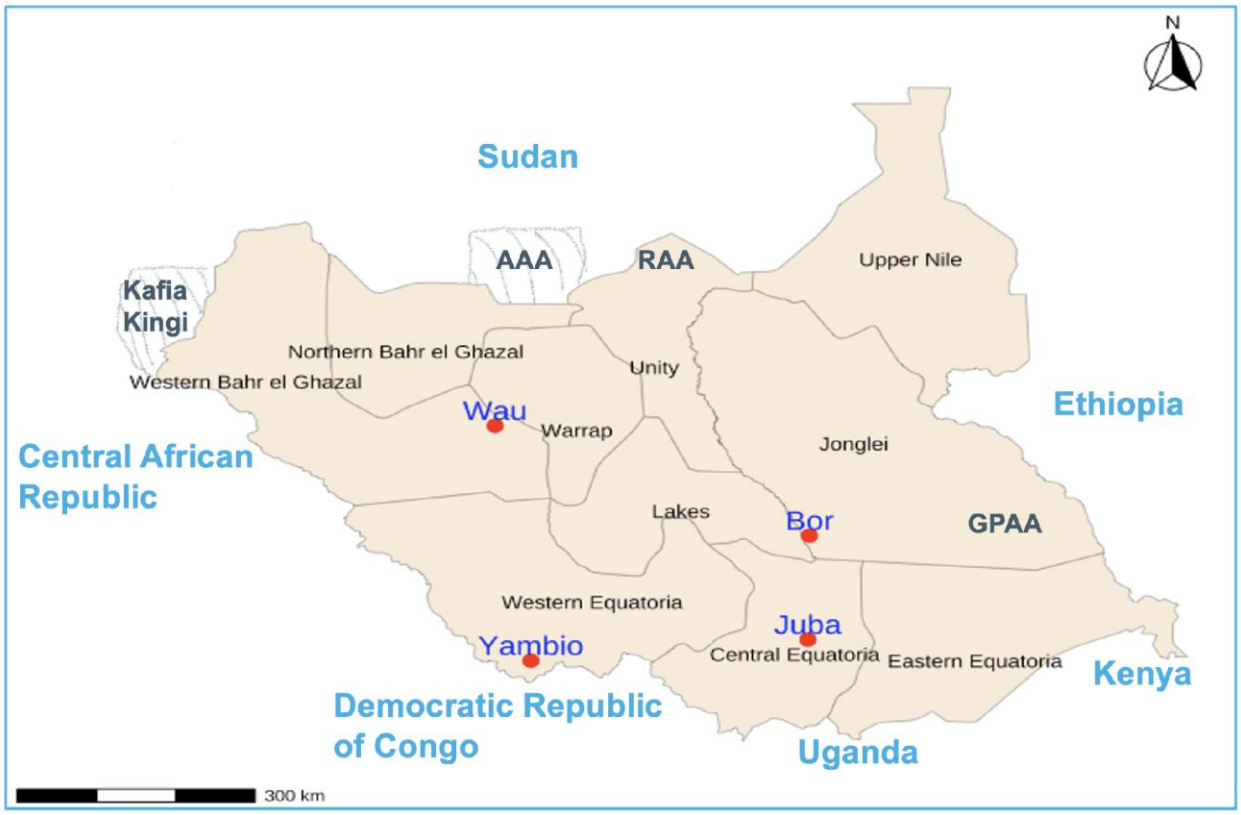
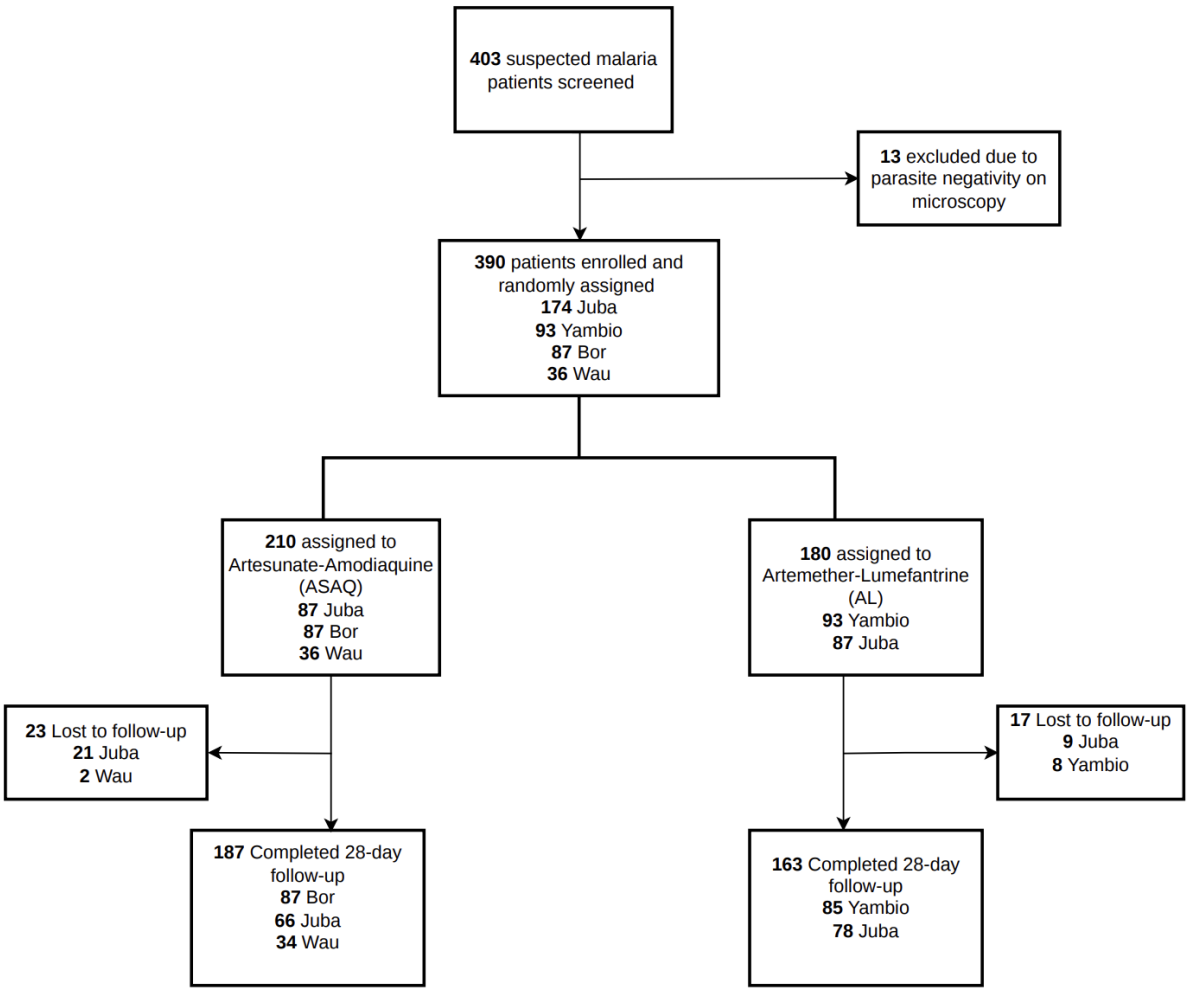


Figure 2. CONSORT flow diagram of participant enrolment and follow-up across all four arms.

651 Numbers of participants screened, enrolled, excluded, lost to follow-up, and included in per-
652 protocol analyses are shown for each arm.

Figure 2: Trial Profile, antimalarial therapeutic efficacy study, South Sudan, 2024-2025

653



654

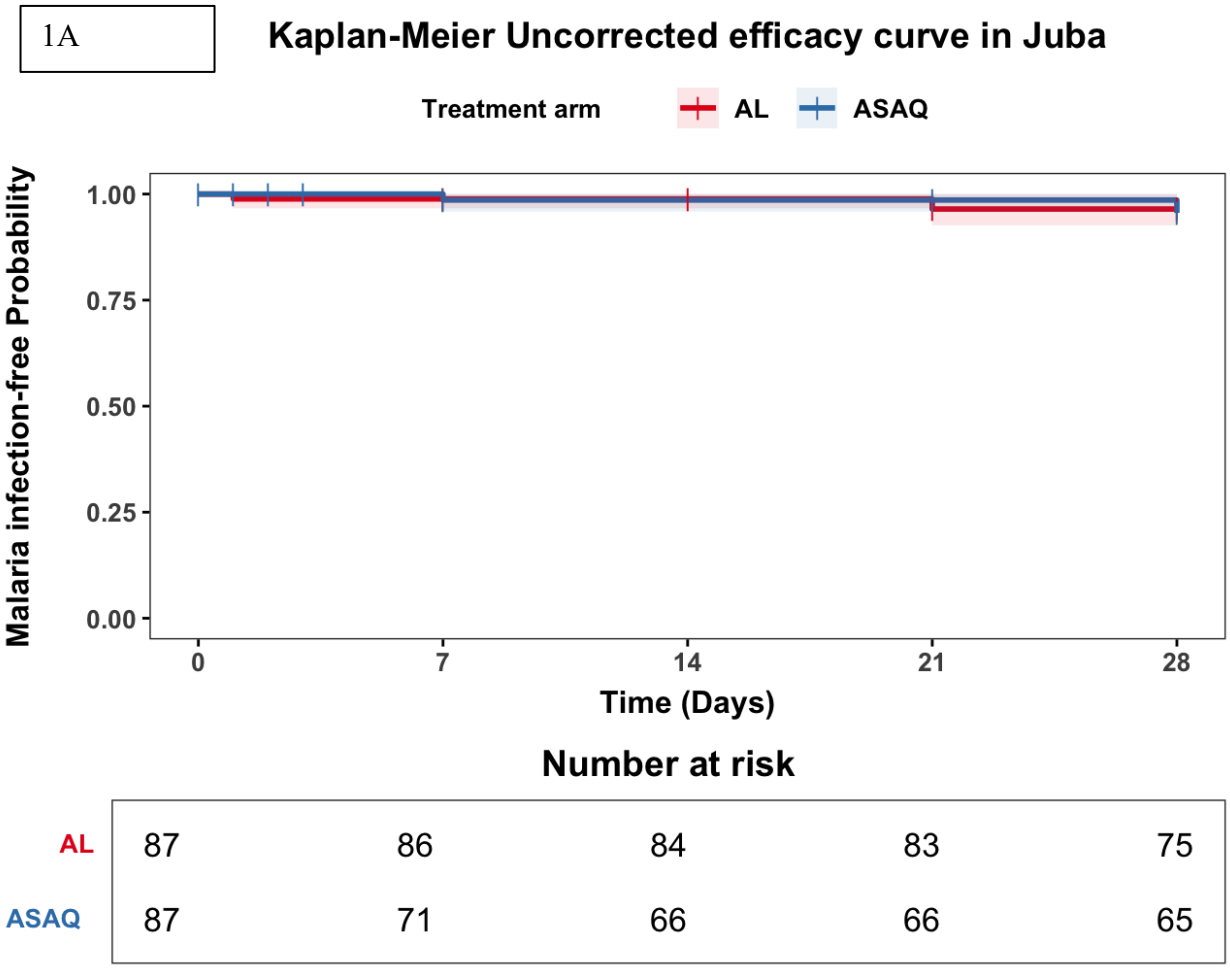
655
656

657

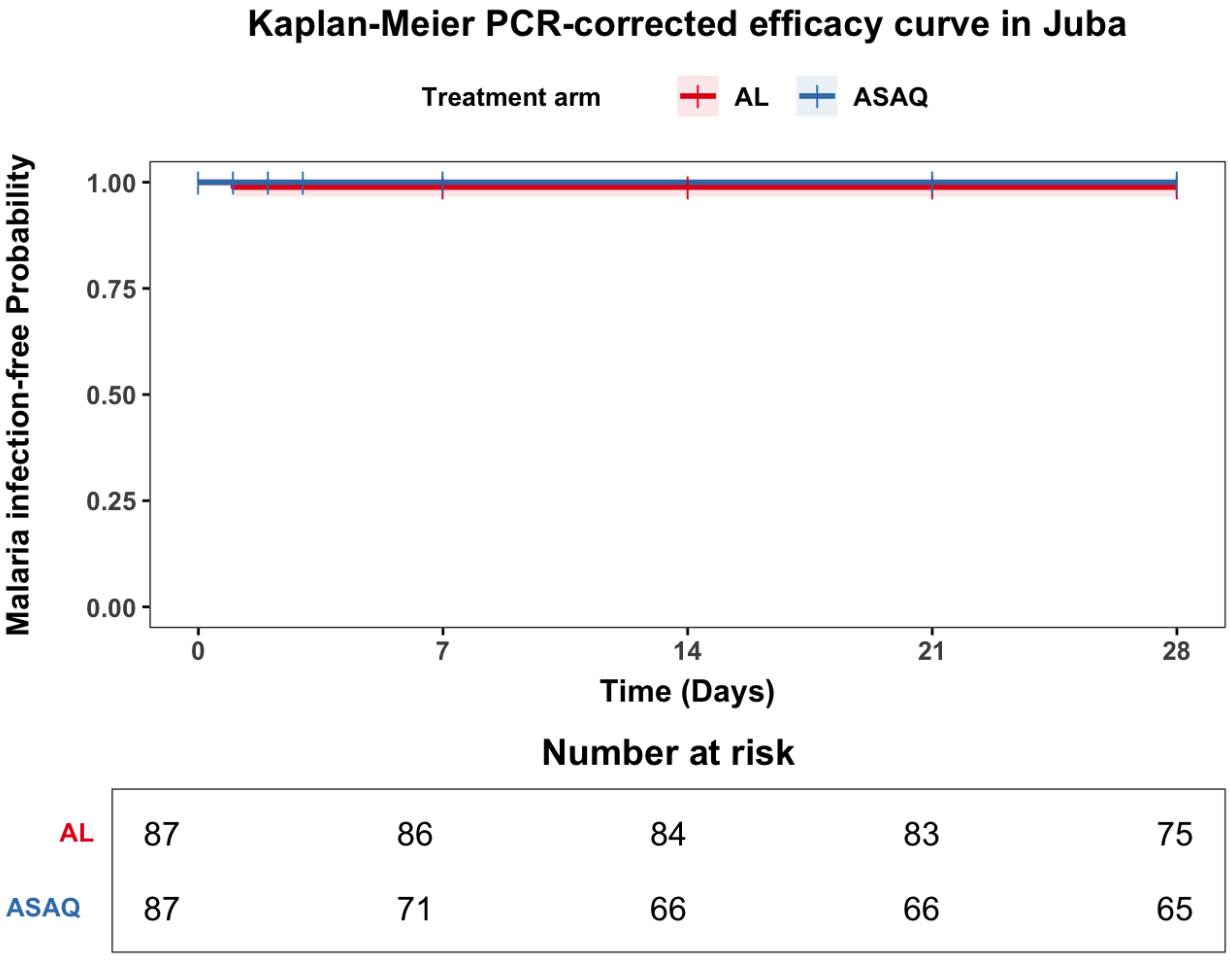
658

Figure 3. Kaplan–Meier survival curves for uncorrected and PCR-corrected adequate clinical and parasitological response (ACPR) by study arm over 28 days of follow-up.

Separate panels are shown for (1) Juba (AL and ASAQ), (2) Bor, (3) Yambio, and (4) Wau. Tick marks represent censored observations. Shaded bands indicate 95% confidence intervals.



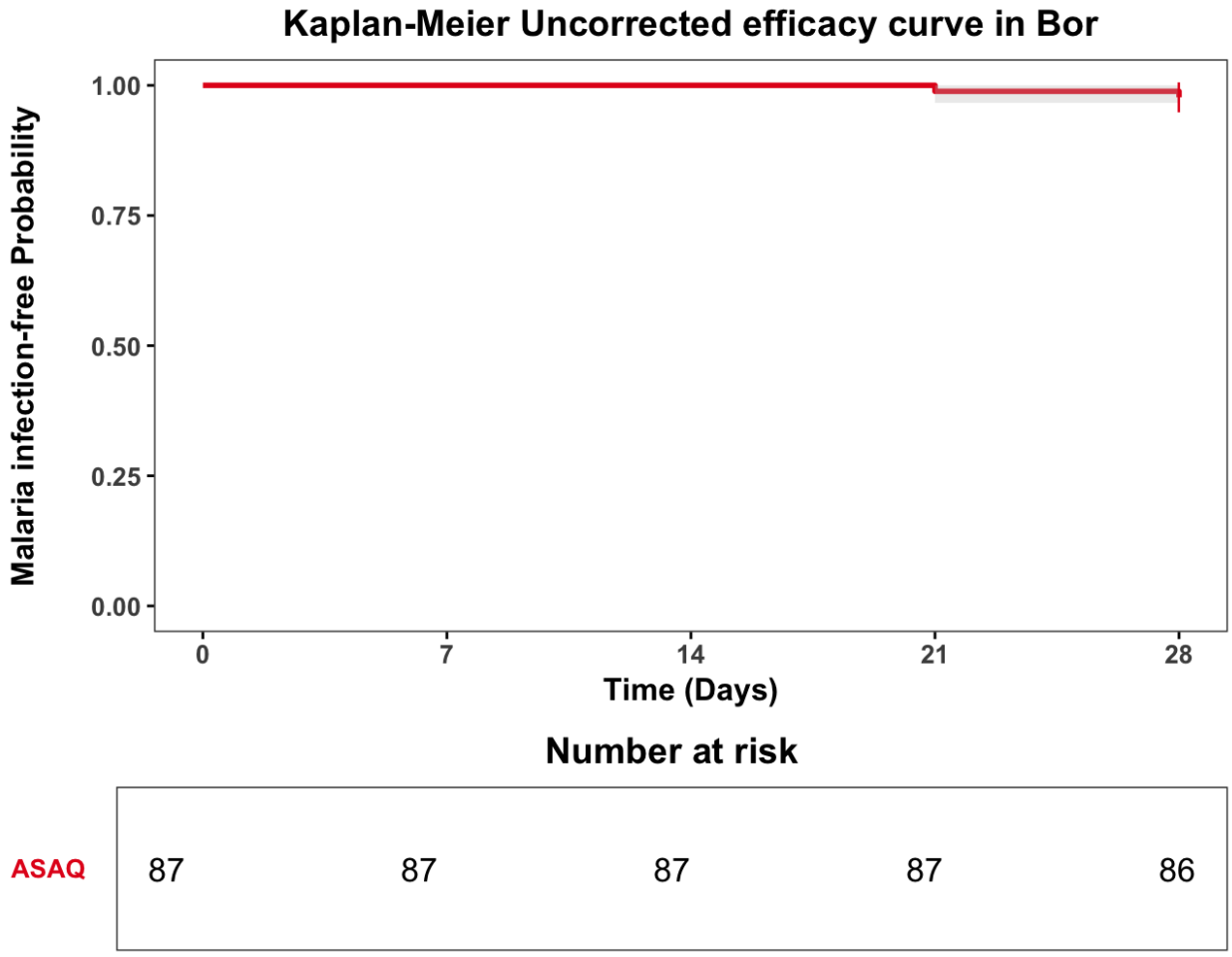
1B


Kaplan-Meier PCR-corrected efficacy curve in Juba
Treatment arm
AL
ASAQ
Malaria infection-free Probability
1.00
0.75
0.50
0.25
0.00
0
7
14
21
28
Time (Days)
Number at risk
AL 87 86 84 83 75
ASAQ 87 71 66 66 65

664

665

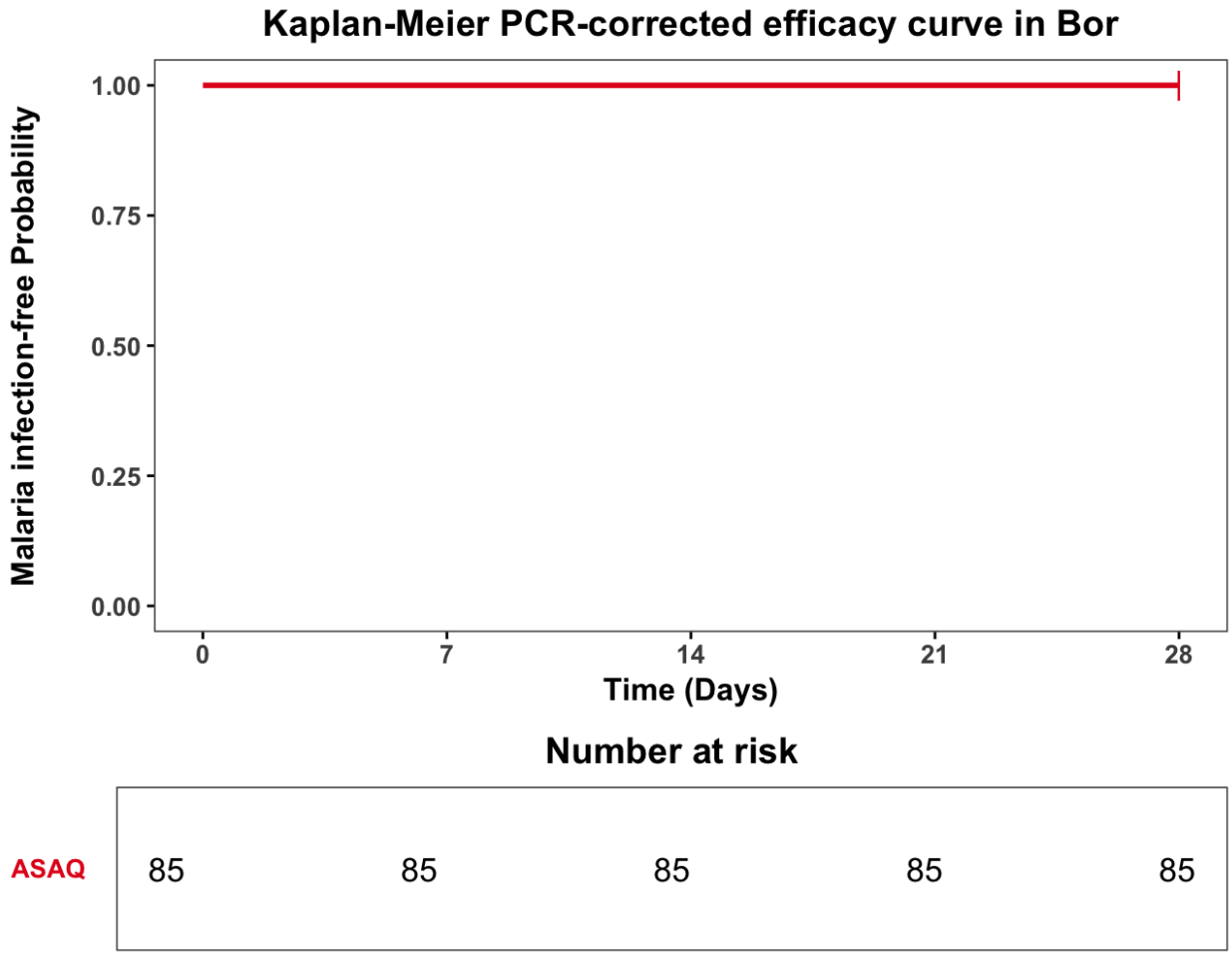
2A


Kaplan-Meier Uncorrected efficacy curve in Bor
Malaria infection-free Probability
1.00
0.75
0.50
0.25
0.00
0
7
14
21
28
Time (Days)
Number at risk
ASAQ
87
87
87
87
86

666

667

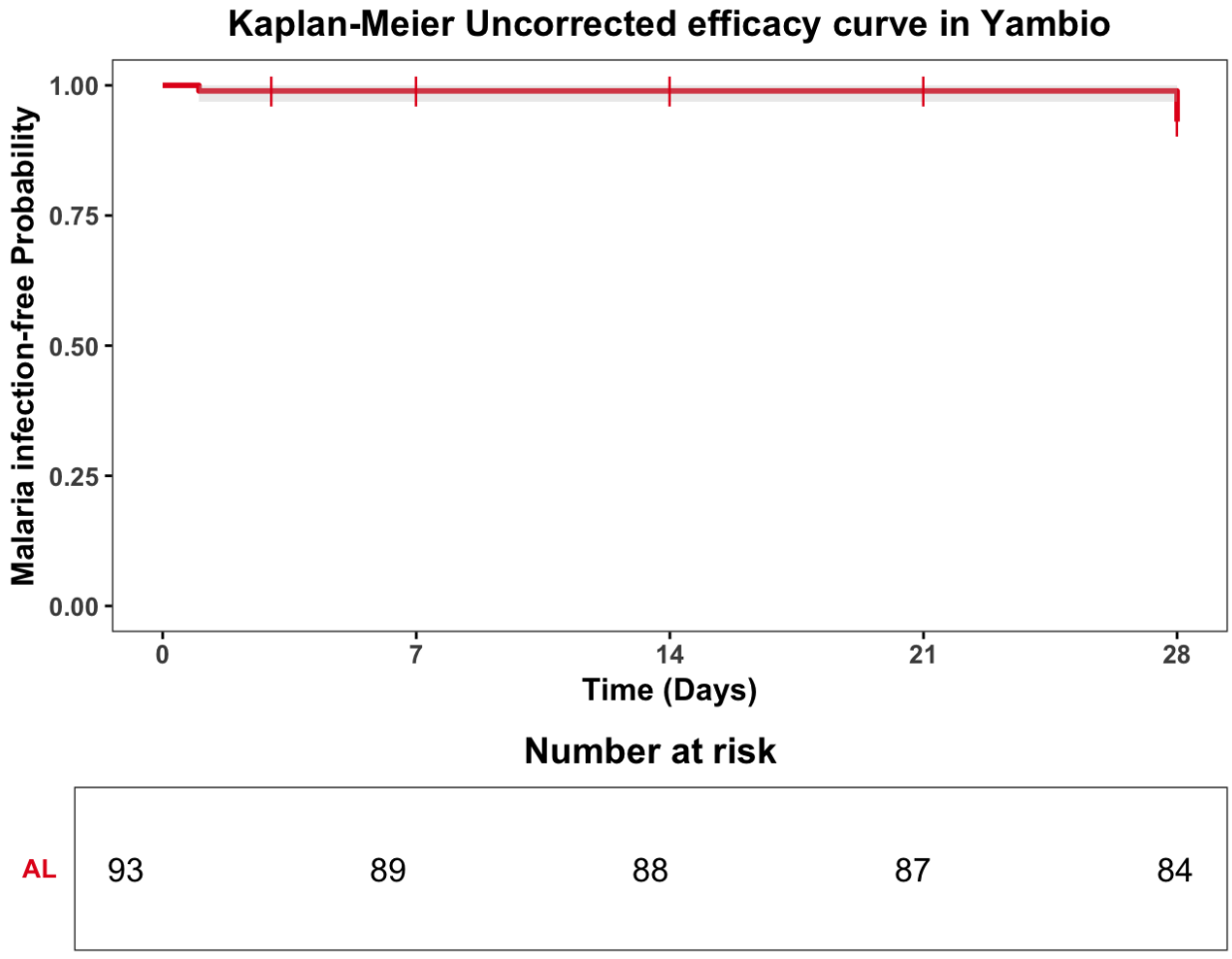
2B


Kaplan-Meier PCR-corrected efficacy curve in Bor
Malaria infection-free Probability
1.00
0.75
0.50
0.25
0.00
0
7
14
21
28
Time (Days)
Number at risk
ASAQ
85
85
85
85
85

668

669

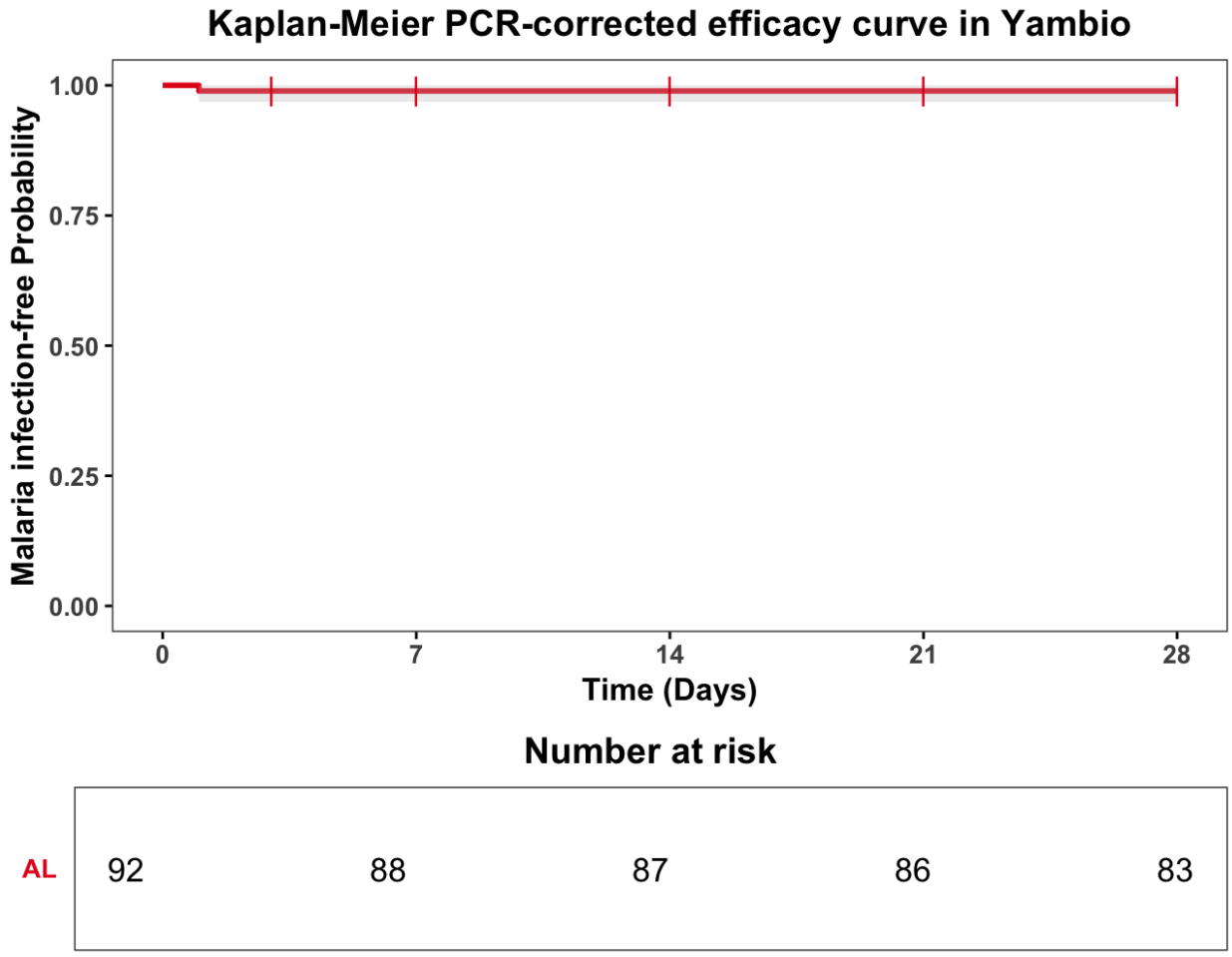
3A


Kaplan-Meier Uncorrected efficacy curve in Yambio
Malaria infection-free Probability
1.00
0.75
0.50
0.25
0.00
0
7
14
21
28
Time (Days)
Number at risk
AL
93
89
88
87
84

670

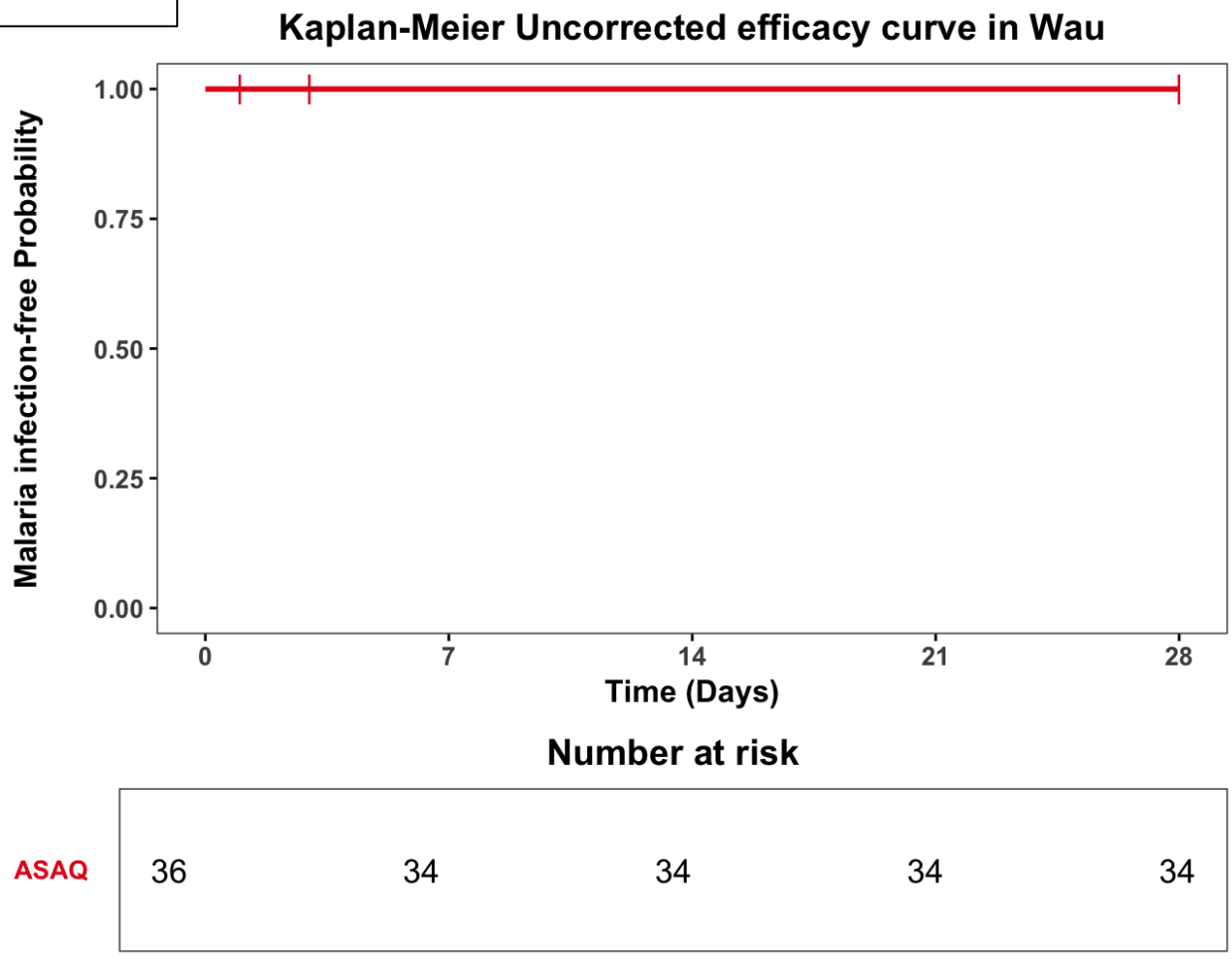
671

3B


Kaplan-Meier PCR-corrected efficacy curve in Yambio
Malaria infection-free Probability
1.00
0.75
0.50
0.25
0.00
0
7
14
21
28
Time (Days)
Number at risk
AL
92
88
87
86
83

672

4A

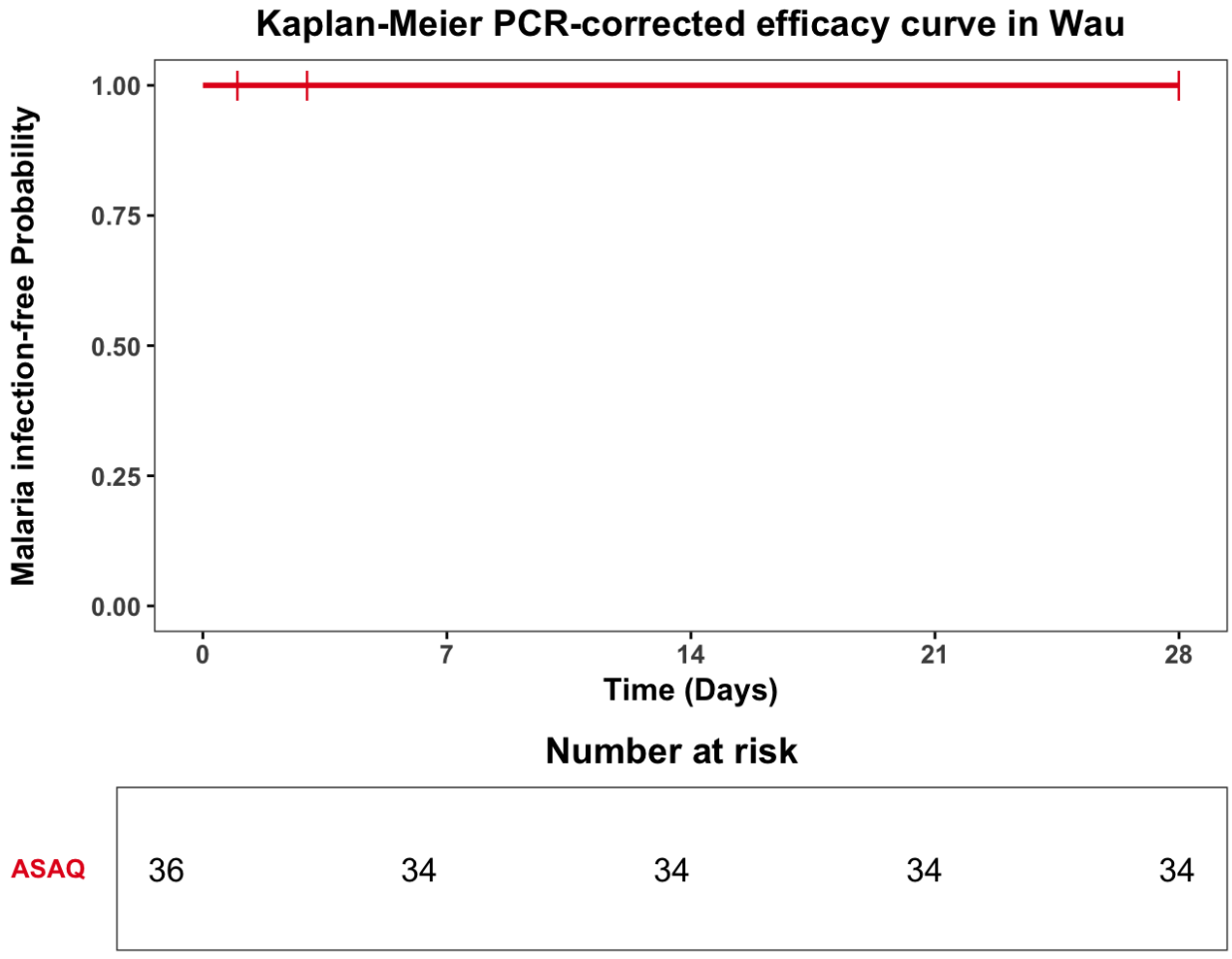


673

674

675

4B



676

677

678

679

680

681

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 2.SSDTESdrugdosing.docx