THE LANCET
Infectious Diseases

Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kamya MR, Nankabirwa JI, Ebong C, 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* 2025; published online Aug 19. https://doi.org/10.1016/S1473-3099(25)00407-4.

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Table A1: Prevalence of PfK13 mutations at baseline and comparison by study site

Variants of PfK13	Prevalence			Comparisons between Sites (p-value)		
	Agago n (col %)	Arua n (col %)	Busia n (col %)	Agago vs. Arua	Agago vs. Busia	Arua vs. Busia
Cys469Tyr	73/252(29%)	5/237(2%)	1/265(0%)	<0·0001[c]	<0·0001[f]	0·11[f]
Ala569Ser	1/252(0%)	1/237(0%)	0/265(0%)	>0·99[f]	0·49[f]	0·47[f]
Ala578Ser	0/252(0%)	3/237(1%)	4/265(2%)	0·11[f]	0·12[f]	>0·99[f]
Cys580Tyr	0/252(0%)	1/237(0%)	0/265(0%)	0·48[f]	NA	0·47[f]
Ala675Val	14/251(6%)	13/236(6%)	6/265(2%)	0·97[c]	0·051[c]	0·058[c]

[c] = Chi-square test; [f] = Fisher's exact test

Table A2: Association between baseline PfK13 mutations and parasite clearance

Variable	PfK13 WT[1]	PfK13 Cys469Tyr	p-value	PfK13 Ala675Val	p-value
Day 3 parasitaemia, n (%)	19/638(3%)	7/79(9%)	0·018[c]	7/33(21%)	0·0001[c]
Parasite clearance rate, Median (IQR)	2·8 (2·3 – 3·6)	4·2 (3·4 – 4·9)	<0·0001[m]	4·9 (3·4 – 5·7)	<0·0001[m]
Half-lives >5·5hrs, n (%)	33/637(5%)	16/79(20%)	<0·0001[c]	9/32(28%)	0·0001[c]

[1]WT = Wild type; [c] = Chi-square test; [m] = Mann-Whitney U test

Table A3: Ex vivo drug susceptibility results of isolates from Agago and Busia Districts

Drug	IC_{50} (nM)	
	Agago (n=27)	Busia (n=69)
Lumefantrine	14·0 (10·4 – 19·4)	10·0 (7·3 – 17·3)
MDAQ[1]	9·3(4·4 – 11·2)	8·4(4·4 – 11·0)
Piperaquine	5·3 (4·8 – 6·2)	7·2 (5·5 – 9·0)
Pyronaridine	1·0 (0·3 – 1·9)	2·2 (1·6 – 3·9)
DHA[1]	2·4 (1·8 – 3·9)	3·8 (2·3 – 5·3)
	RSA (%)	
	Agago (n=30)	Busia (n=47)
DHA[1]	2·7 (1·1 – 7·6)	5·4 (2·2 – 18·7)

[1] MDAQ = Monodesethyloamodiaquine, DHA = Dihydroartemisinin; RSA= Ring-stage Survival Assay; IC_{50} = Half-Maximal Inhibitory Concentration

Table A4: Day-7 lumefantrine blood levels and their comparison by site and treatment outcomes.

	Lumefantrine blood levels	P-value
Overall		
Total	478	
Overall, Median (IQR)	383·5(237·0 – 614·0)	NA
Below 200 ng/Ml, n (%)	96/478(20·1%)	NA
Treatment outcome	**Lumefantrine levels below 200ng/Ml**	
ACPR, n (%)	61/290(21%)	0.52[c]
Recurrent Malaria, n (%)	35/188(19%)	
Comparisons between sites	**Lumefantrine levels below 200ng/Ml**	
Agago, n (%)	27/157(17%)	0·82[c]
Arua,, n (%)	26/160(16%)	
Agago, n (%)	27/157(17%)	0.041[c]
Busia, n (%)	43/161(27%)	
Arua, n (%)	26/160(16%)	0.023[c]
Busia, n (%)	43/161(27%)	

[c] = Chi-square test; [f] = Fisher's exact test; ACPR = Adequate Clinical and Parasitological Response;

IQR = Interquartile Range

Table A5: Treatment outcomes stratified by age group and PfK13 mutations status

Variable	ACPR	Recurrent malaria	P-value
Age categories			
<5 years	324/571(57%)	128/226(57%)	0.98[c]
5-10 years	247/571(43%)	98/226(43%)	
PK13 mutations			
PfK13 Cys469Tyr			
Mutant	69/549(13%)	8/194(4%)	0.0009[c]
Wild type	480/549(87%)	186/194(96%)	
Pfk13 Cys580Tyr			
Mutant	1/549(0%)	0/194(0%)	>0.99[f]
Wild type	548/549(100%)	194/194(100%)	
Pfk13 Ala675Val			
Mutant	23/547(4%)	10/194(5%)	0.58[c]
Wild type	524/547(96%)	184/194(95%)	
PfK13 Ala578Ser			
Mutant	5/549(1%)	2/194(1%)	>0.99[f]
Wild type	544/549(99%)	192/194(99%)	
PfK13 Ala569Ser			
Mutant	0/549(0%)	2/194(1%)	0.068[f]
Wild type	549/549(100%)	192/194(99%)	

[c] = Chi-square test; [f] = Fisher's exact test; ACPR = Adequate Clinical and Parasitological Response

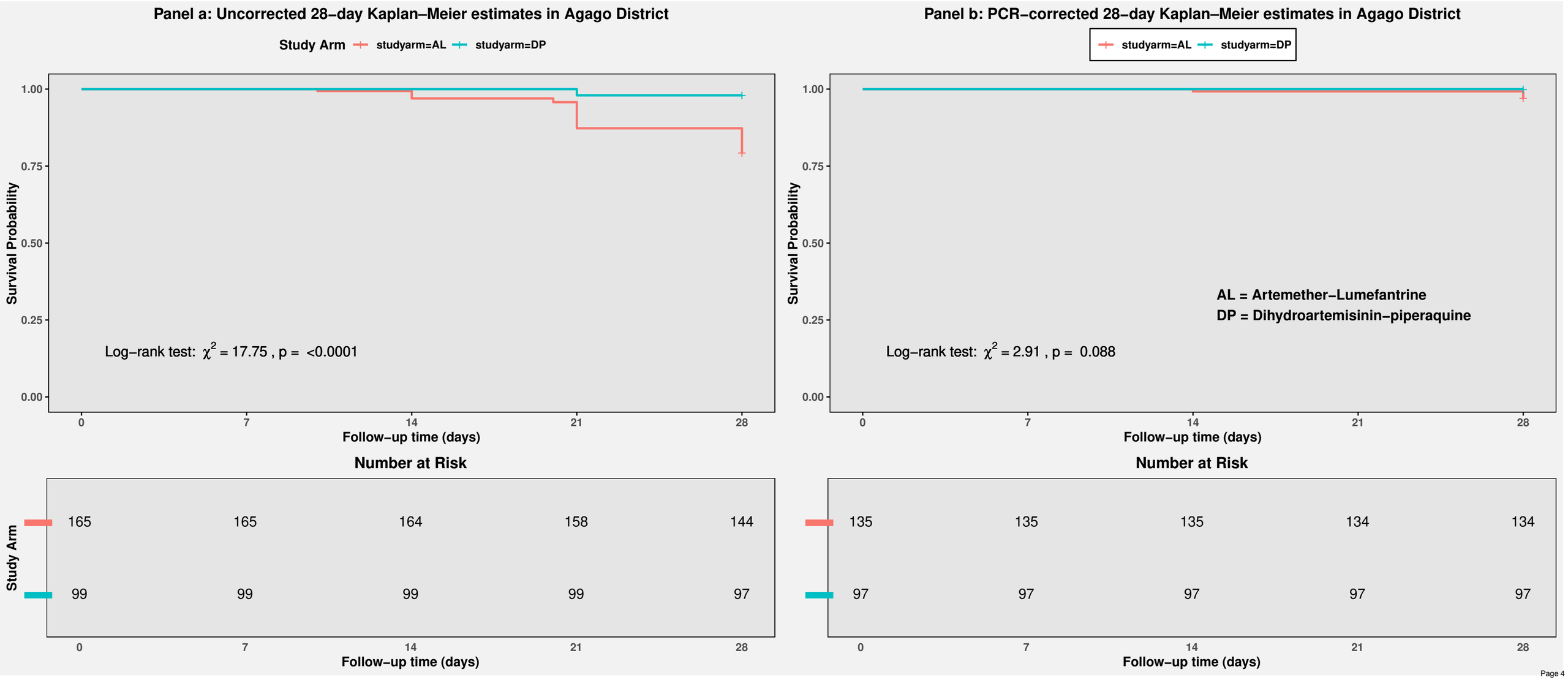
Table A6: Comparison of PCR-corrected efficacies using 2/3 vs. 3/3 genotyping match algorithms

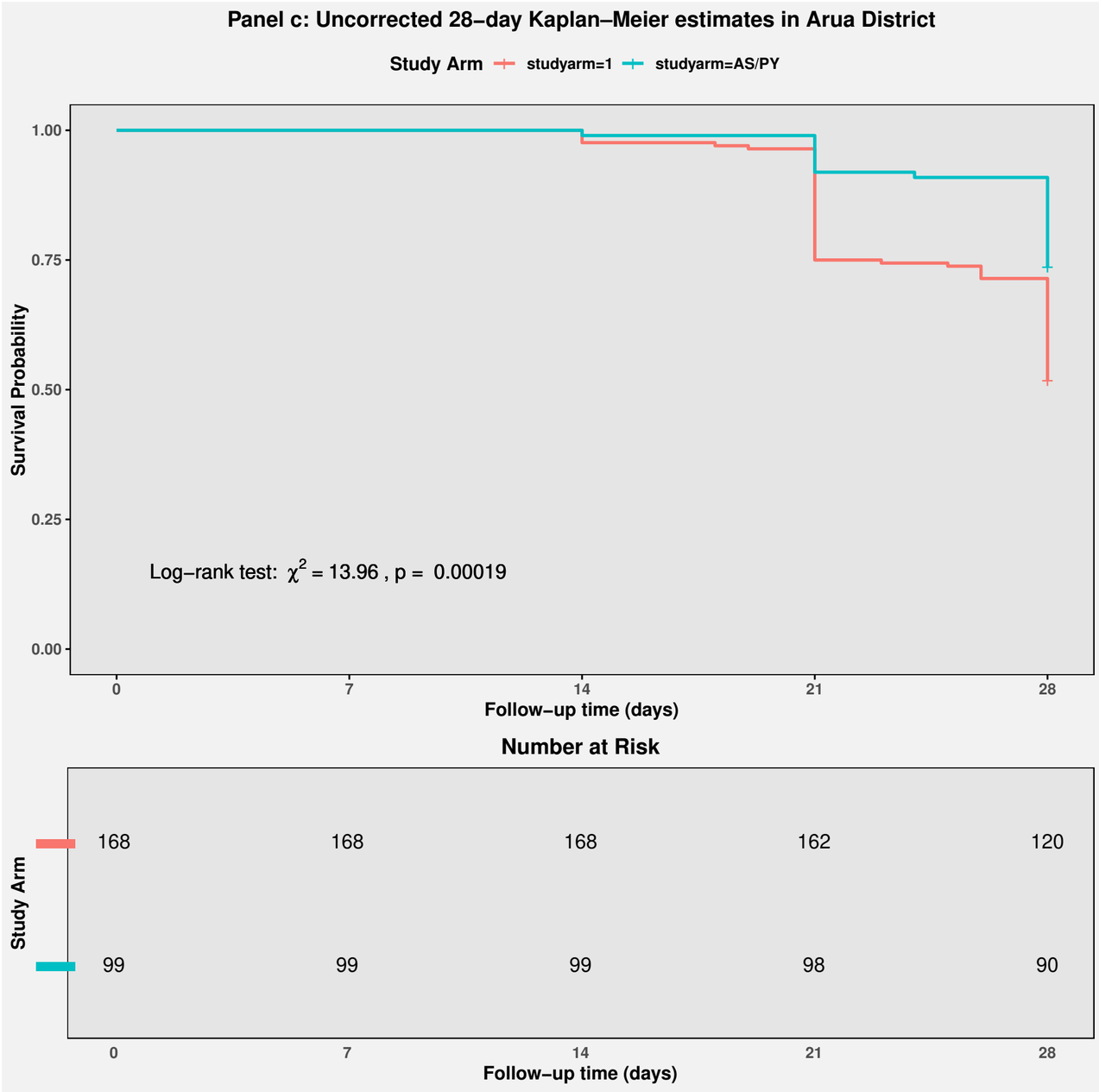
Outcome	Agago			Arua			Busia	
	AL[1] (28 Days)	DP[1] (28 Days)	DP[1] (42 Days)	AL[1] (28 Days)	AS/PY[1] (28 Days)	AS/PY[1] (42 Days)	AL[1] (28 Days)	AS/AQ[1] (28 Days)
Corrected outcomes (3/3 match algorithm)								
PCR[2] corrected efficacy, % (95% CI)	131/135(97·0 %) (92·1% – 99·0%)	97/97(100·0%) (95·3% – 100·0%)	80/84(95·2%) (87·6% – 98·5%)	87/106(82·1%) (73·2% – 88·6%)	73/79(92·4%) (83·6% – 96·9%)	54/62(87·1%) (75·6% – 93·9%)	88/108(81·5%) (72·6% – 88·1%)	95/95(100·0%) (95·2% – 100·0%)
PCR[2] corrected efficacy, KM[3] estimates (95% CI)	97·0 (94·2 – 100·0)	100·0 (100·0 – 100·0)	95·2 (90·8 – 99·9)	82·1 (75·1 – 89·7)	92·4 (86·7 – 98·4)	87·1 (79·1 – 95·9)	81·5 (74·5 – 89·1)	100·0 (100·0 – 100·0)
Corrected outcomes (2/3 match algorithm)								
PCR[2] corrected efficacy, % (95% CI)	130/139(93·5 %) (87·7% – 96·8%)	97/98(99·0%) (93·6% – 99·9%)	79/83(95·2) (87·5 – 98·4)	84/107(78·5%) (69·3% – 85·6%)	73/86(84·9%) (75·2% – 91·4%)	53/72(73·6) (61·7 – 83·0)	87/125(69·6%) (60·6% – 77·3%)	94/94(100·0%) (95·1% – 100·0%)
PCR[2] corrected efficacy, KM[3] estimates (95% CI)	93.5 (89·5 – 97·7)	99·0 (97·1 – 100·0)	95.2 (90·7 – 99·9)	78·5 (71·1 – 86·7)	84.9 (77·6 – 92·8)	73.6 (64·1 – 84·5)	69·6 (62·0 – 78·0)	100·0 (100·0 – 100·0)

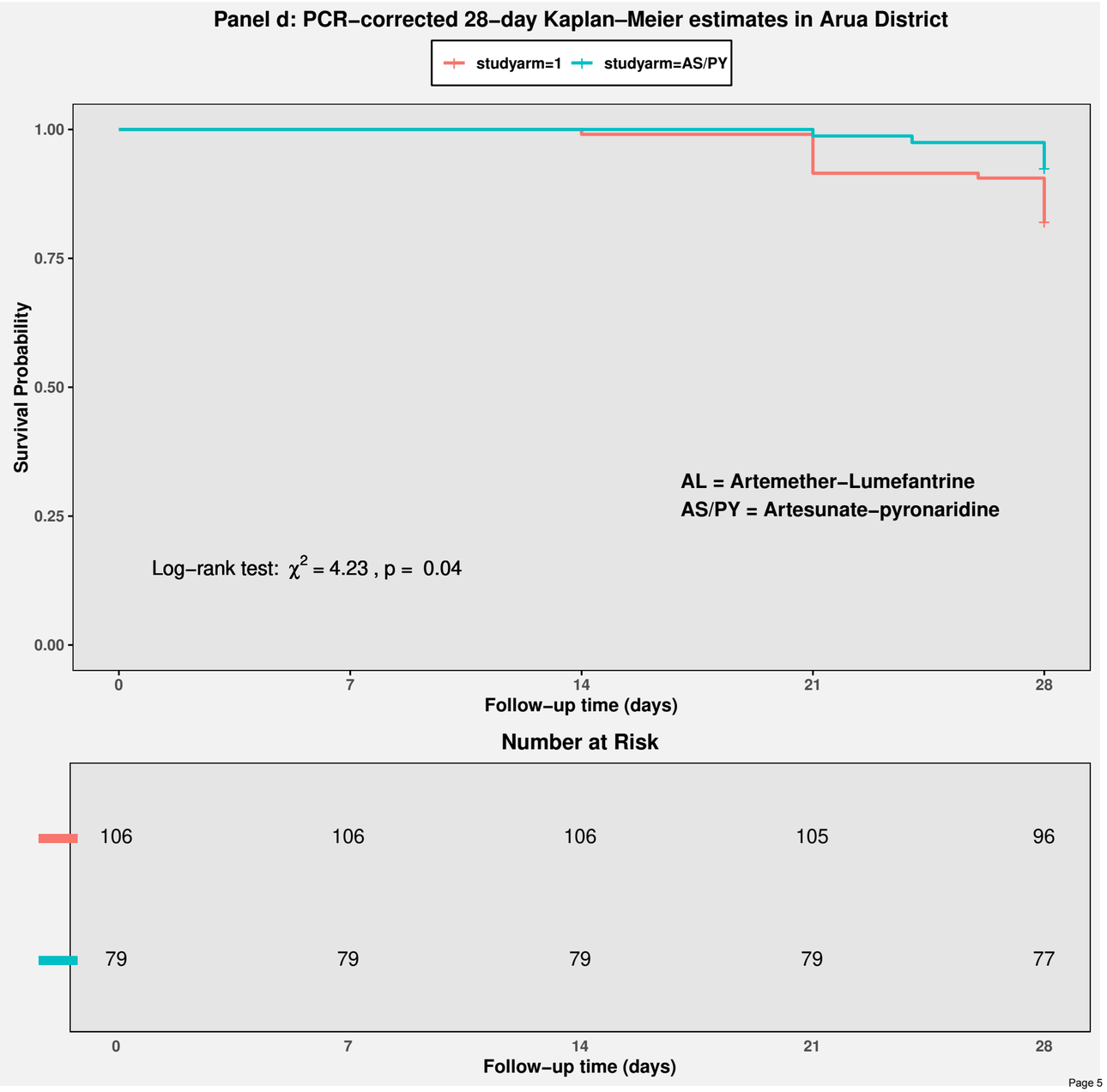
[1]AL = Artemether-Lumefantrine, DP = Dihydroartemisinin-piperaquine, AS/PY = Artesunate-pyronaridine, ASAQ = Artesunate-amodiaquine;

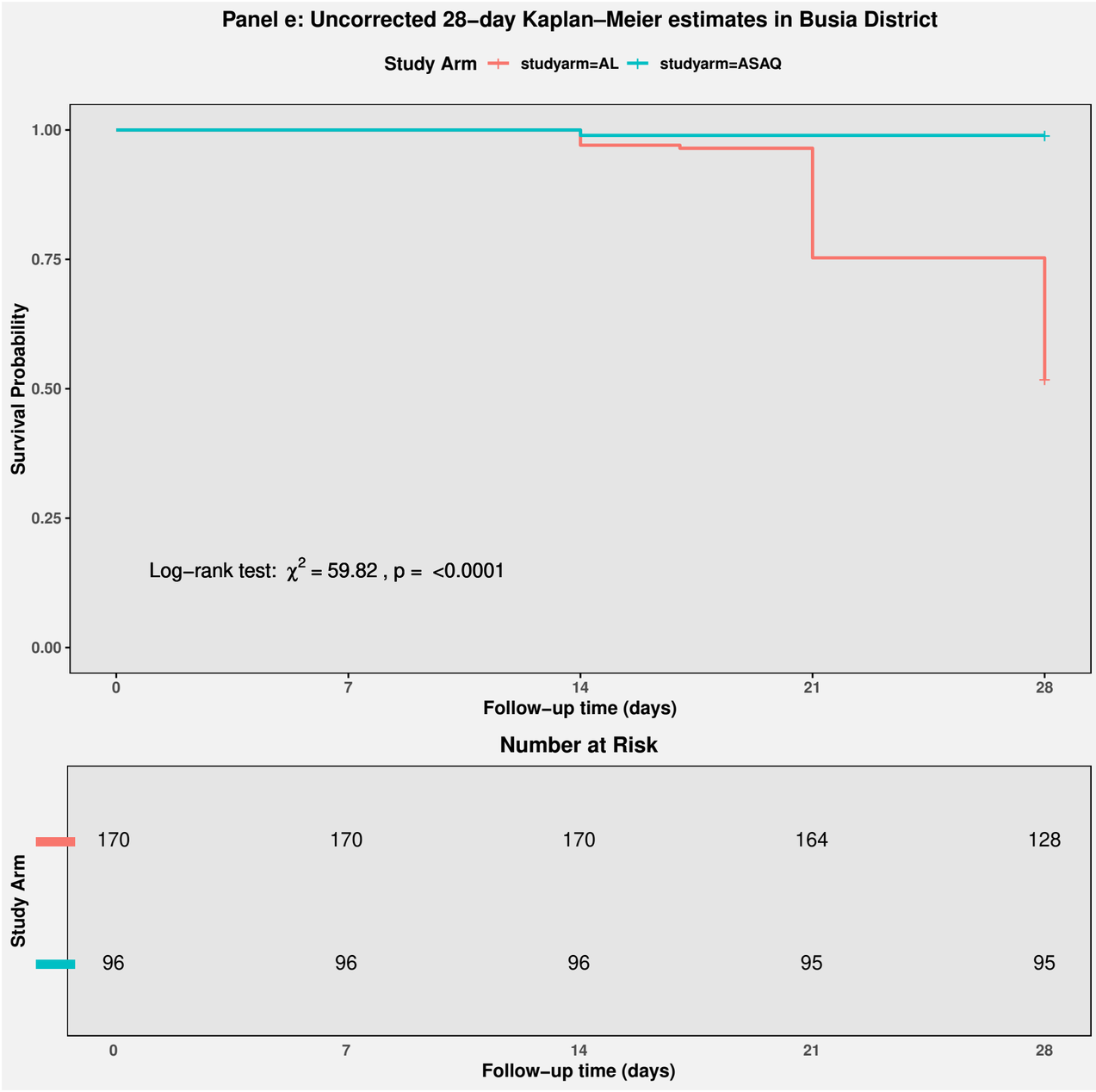
[2]PCR = Polymerase Chain Reaction; [3]KM = Kaplan Meier

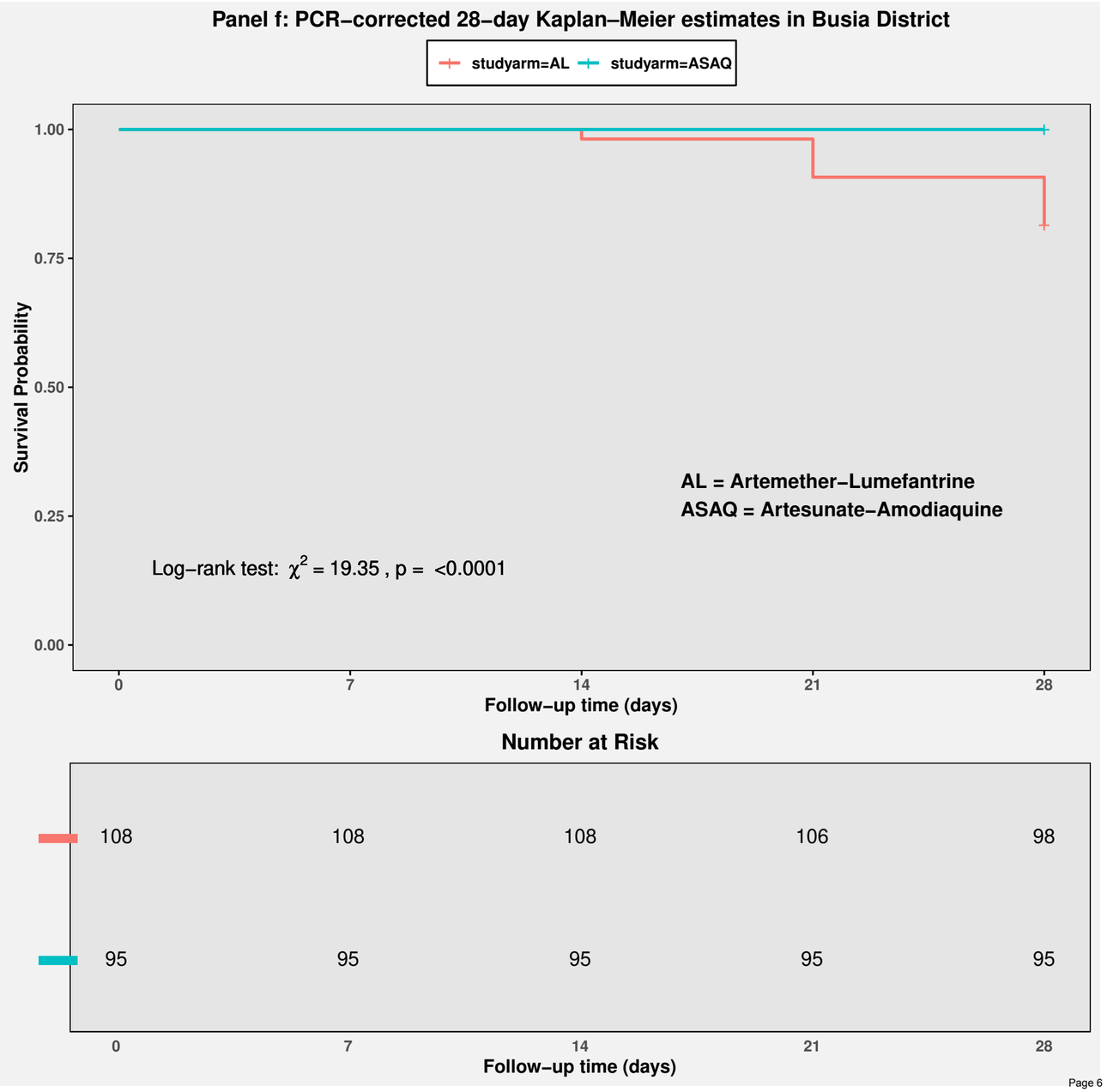
Figure A1: Kaplan–Meier survival analysis of uncorrected and corrected outcomes at 28 days




Panel c: Uncorrected 28−day Kaplan−Meier estimates in Arua District
Study Arm
studyarm=1
studyarm=AS/PY
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log−rank test: χ^2 = 13.96 , p = 0.00019
0
7
14
21
28
Follow−up time (days)
Number at Risk
Study Arm
168
168
168
162
120
99
99
99
98
90
0
7
14
21
28
Follow−up time (days)


Panel d: PCR−corrected 28−day Kaplan−Meier estimates in Arua District
studyarm=1
studyarm=AS/PY
Survival Probability
1.00
0.75
0.50
0.25
0.00
AL = Artemether−Lumefantrine
AS/PY = Artesunate−pyronaridine
Log−rank test: χ^2 = 4.23 , p = 0.04
0
7
14
21
28
Follow−up time (days)
Number at Risk
106
106
106
105
96
79
79
79
79
77
0
7
14
21
28
Follow−up time (days)


Panel e: Uncorrected 28−day Kaplan−Meier estimates in Busia District
Study Arm
studyarm=AL
studyarm=ASAQ
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log−rank test: χ^2 = 59.82 , p = <0.0001
0
7
14
21
28
Follow−up time (days)
Number at Risk
Study Arm
170
170
170
164
128
96
96
96
95
95
0
7
14
21
28
Follow−up time (days)


Panel f: PCR−corrected 28−day Kaplan−Meier estimates in Busia District
studyarm=AL
studyarm=ASAQ
Survival Probability
1.00
0.75
0.50
0.25
0.00
AL = Artemether−Lumefantrine
ASAQ = Artesunate−Amodiaquine
Log−rank test: χ^2 = 19.35 , p = <0.0001
0
7
14
21
28
Follow−up time (days)
Number at Risk
108
108
108
106
98
95
95
95
95
95
0
7
14
21
28
Follow−up time (days)

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	1
	1b	Structured summary of the trial design, methods, results, and conclusions	2
Open science			
Trial registration	2	Name of trial registry, identifying number (with URL) and date of registration	6
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	5
Data sharing	4	Where and how the individual de-identified participant data (including data dictionary), statistical code and any other materials can be accessed	14 data sharing
Funding and conflicts of interest	5a	Sources of funding and other support (eg, supply of drugs), and role of funders in the design, conduct, analysis and reporting of the trial	9, 14
	5b	Financial and other conflicts of interest of the manuscript authors	13-14
Introduction			
Background and rationale	6	Scientific background and rationale	5
Objectives	7	Specific objectives related to benefits and harms	5
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	NA (standardized WHO protocol used)
Trial design	9	Description of trial design including type of trial (eg, parallel group, crossover), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	13
Trial setting	11	Settings (eg, community, hospital) and locations (eg, countries, sites) where the trial was conducted	5
Eligibility criteria	12a	Eligibility criteria for participants	6
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions (eg, surgeons, physiotherapists)	NA
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator (eg, intervention manual) can be accessed	6
Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome	7-9
Harms	15	How harms were defined and assessed (eg, systematically, non-systematically)	7
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	8

	16b	Explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	6
	17b	Type of randomisation and details of any restriction (eg, stratification, blocking and block size)	6
Allocation concealment mechanism	18	Mechanism used to implement the random allocation sequence (eg, central computer/telephone; sequentially numbered, opaque, sealed containers), describing any steps to conceal the sequence until interventions were assigned	6
Implementation	19	Whether the personnel who enrolled and those who assigned participants to the interventions had access to the random allocation sequence	6
Blinding	20a	Who was blinded after assignment to interventions (eg, participants, care providers, outcome assessors, data analysts)	NA
	20b	If blinded, how blinding was achieved and description of the similarity of interventions	NA
Statistical methods	21a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	9
	21b	Definition of who is included in each analysis (eg, all randomised participants), and in which group	9
	21c	How missing data were handled in the analysis	9
	21d	Methods for any additional analyses (eg, subgroup and sensitivity analyses), distinguishing prespecified from post hoc	9
Results			
Participant flow, including flow diagram	22a	For each group, the numbers of participants who were randomly assigned, received intended intervention, and were analysed for the primary outcome	9
	22b	For each group, losses and exclusions after randomisation, together with reasons	Flow diagram
Recruitment	23a	Dates defining the periods of recruitment and follow-up for outcomes of benefits and harms	9
	23b	If relevant, why the trial ended or was stopped	NA
Intervention and comparator delivery	24a	Intervention and comparator as they were actually administered (eg, where appropriate, who delivered the intervention/comparator, how participants adhered, whether they were delivered as intended (fidelity))	6
	24b	Concomitant care received during the trial for each group	6
Baseline data	25	A table showing baseline demographic and clinical characteristics for each group	Table 1, Page 16
Numbers analysed, outcomes and estimation	26	For each primary and secondary outcome, by group: ● the number of participants included in the analysis ● the number of participants with available data at the outcome time point ● result for each group, and the estimated effect size and its precision (such as 95% confidence interval) ● for binary outcomes, presentation of both absolute and relative effect size	Tables 2 and 3; Page 17 and 18
Harms	27	All harms or unintended events in each group	Table 4, Page 18
Ancillary analyses	28	Any other analyses performed, including subgroup and sensitivity analyses, distinguishing pre-specified from post hoc	Page 10, Table 3,

			supplemental tables
Discussion			
Interpretation	29	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11 - 13
Limitations	30	Trial limitations, addressing sources of potential bias, imprecision, generalisability, and, if relevant, multiplicity of analyses	13

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123
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*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.

THE REPUBLIC OF UGANDA
Ministry of Health

USAID
FROM THE AMERICAN PEOPLE
CDC
U.S. President's Malaria Initiative

EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE, PYRONARIDINE -ARTESUNATE, ARTESUNATE-AMODIAQUINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN IN UGANDA

Protocol version 1.11. dated 18th March 2021

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Title	Efficacy and safety of artemether-lumefantrine, pyronaridine- Artesunate, Artesunate-amodiaquine and dihydroartemisinin-piperaquine for the treatment of uncomplicated plasmodium falciparum malaria in children in Uganda
Study site(s)	Site 1: Moroto Regional referral Hospital, Moroto district Site 2: Masafu Hospital, Busia district Site 3: Oli Health Centre, Arua district Site 4: Kihihi Health centre, Kanungu district
Protocol number	Version 1.8. dated 4th January 2021
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SYNOPSIS

Title: Efficacy and safety of artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin – piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Uganda.

Purpose: To assess the efficacy of the currently recommended artemisinin-based combination therapies;

Objective: To assess the efficacy and safety of artemether-lumefantrine, pyronaridine-artesunate artesunate-amodiaquine and dihydroartemisinin – piperaquine for the treatment of uncomplicated *P. falciparum* malaria.

Study Sites: Masafu hospital in Busia district, Oli Health Centre IV in Arua district, Kihihi health centre IV in Kanungu district and Moroto Regional Referral Hospital in Moroto District.

Study Period: February 2021 to September 2022.

Study Design: This is an open label randomized clinical trial at four sentinel sites.

Patient population: Febrile patients aged 6 months to 10 years with confirmed uncomplicated *P. falciparum* malaria.

Sample Size: 200 patients for AL treatment arm and 100 patients for the other treatment arms per site. At least two drugs will be studied per site.

Treatment(s) and follow-up: Participants will be randomized to artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin – piperaquine. At least 2 drugs will be tested per site. The choice of the drugs to be tested will be decided by the Ministry of Health. Clinical and parasitological parameters will be monitored over a 28-day follow-up for patients treated with artemether-lumefantrine and artesunate-amodiaquine and over 42 day period for patients treated with pyronaridine-artesunate and Dihydroartemisinin – piperaquine.

Primary endpoints: The proportion of patients with early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response as indicators of efficacy. Recrudescence will be distinguished from re-infection by using molecular techniques.

Secondary endpoints: The frequency and nature of adverse events; prevalence of molecular markers of drug resistance, Day7 blood concentration of Lumefantrine.

1. 0 BACKGROUND

Malaria remains a leading cause of childhood illness and death worldwide causing an estimated 212 million cases and 429 000 deaths. About 90% of the cases and deaths are in sub -Saharan Africa and in children aged under 5 years [1]. Uganda has the third highest number of *P. falciparum* infections in sub-Saharan Africa, and has some of the highest reported malaria transmission rates in the world [2]. Significant progress in malaria control has been realized in the last decade due to increased access to proven interventions, including long lasting insecticidal nets (LLINs), indoor residual spraying of insecticides (IRS), effective case management with artemisinin-based combination therapies (ACTs), and intermittent preventive therapy (IPT) for high-risk groups [1]. With changing epidemiology, reliable surveillance is essential for detecting the emergence of resistance to drug or vector control interventions.

Early, effective treatment is the cornerstone of malaria control. Most malaria endemic countries have adopted ACTs as the first-line treatment for *P. falciparum* malaria, as recommended by the World Health Organisation (WHO) [3]. The ACTs recommended for the treatment of uncomplicated malaria are artemether–lumefantrine (AL), artesunate–amodiaquine (AS+AQ), artesunate–mefloquine (AS+MQ), artesunate–sulfadoxine–pyrimethamine (AS+SP), dihydroartemisinin–piperaquine (DP) and pyronaridine artesunate (PA) [3]. Resistance against commonly used ACTs is rising in South-East Asia and the potential spread to African countries is a major concern [4, 5]. Pyronaridine-artesunate (PA) is a new ACT developed by Shin Poong Pharmaceuticals (South Korea), in partner-ship with Medicines for Malaria Venture (MMV) [6]. PA was added to the WHO list of prequalified medicinal products in 2017 [7] and has been registered with many national regulatory authorities including Uganda.

Achieving adequate concentrations of antimalarial drugs in blood is valuable in evaluating response to therapy. Accurate measurement of plasma concentrations of antimalarial drugs guides adequate dosing and facilitates differentiation of treatment failure due to inadequate exposure to a drug from failure due to drug resistance. Prior studies have shown that antimalarial concentration at day 7 after therapy and area under the curve are strongly associated with treatment failure, while drug levels at the time of recurrent malaria have been associated with markers of decreased sensitivity to antimalarial drugs [10-12]. Measurement of antimalarial concentrations also aids in identifying populations at high risk of treatment failure who may benefit from dose optimization [13, 14].

The appropriate selection of first- and second-line antimalarial medicines and updating treatment strategies and policies for country programs should be based on the efficacy and safety of the medicines against the malaria parasites and should be guided by the results of therapeutic efficacy studies (TES). The efficacy of national first- and second line antimalarial treatments should be monitored at least once every 2 years, and should be conducted in the

same regions of the country over time in order to allow analysis of trends [8]. According to WHO policy, a change in an antimalarial medicine recommended in the national malaria treatment policy should be initiated if the percentage of genotype-corrected treatment failure is ≥ 10%, as assessed by TES [9]. Routine monitoring of the efficacy of antimalarial drugs is necessary for effective case management and early detection of resistance. It is important for the country to continuously assess the efficacy and safety of its first- and second-line regimens to detect early development of resistance to these drugs. Therefore, we will conduct this study as part of the routine monitoring of the efficacy of antimalarial drugs to guide treatment guideline in Uganda.

1.1 Antimalarial drug efficacy and resistance in Uganda

Antimalarial drug efficacy studies have been conducted in Uganda since 1998 to date. The primary purpose of these studies is to provide data to help guide policy through standardized evaluation of the safety and efficacy of current and new antimalarial regimens being considered for use in Uganda. These studies over the last decade have documented high failure rates with widely used monotherapies, including chloroquine (CQ), amodiaquine (AQ), and sulfadoxine-pyrimethamine (SP) [15, 16]. Early studies with combination antimalarial therapies documented unacceptably high failure rates with the combination of CQ plus SP, which was the recommended first-line treatment for Uganda from 2001-2004 and also with the ACT artesunate-SP [17, 18]. Consistent with these findings, in vitro resistance to CQ and AQ was common among parasites collected in Kampala [19, 20] and the prevalence of molecular markers indicative of resistance to CQ (*pfcrt* 76T), AQ (*pfcrt* 76T, *pfmdr1* 86Y and 1246Y), and SP (5 mutations in *pfdhfr* and *pfdhps*) were all very high [21]. In 2004 the first-line therapy was changed to ACTs, with AL adopted as first line and AS+AQ as an alternative. AL still remains the first line treatment with AS+AQ as an alternative first-line regimen. DP is the second line treatment with quinine used as an alternative and for treatment of malaria during the first trimester of pregnancy[2].

Studies conducted in Uganda showed that AL was significantly superior to the combinations of amodiaquine-SP and of comparable efficacy to AS+AQ [15]. DP and AS+AQ have also performed very well in recent trials [22-25]. The efficacy of AL, DP and AS + AQ is summarised in table 1.

Table 1. Results of antimalarial drug surveillance studies in Uganda.

Year and reference	*Study site*	*Duration of follow-up*	*Treatment arms*[*]	*Number enrolled*	*Risk of treatment failure*	
					Unadjusted by genotyping	Adjusted by genotyping
2003	Kampala	28 days	AS+AQ	134	18%	3%
2003	Jinja	28 days	AS+AQ	189	19%	4%
2004	Apac	28 days	AS+AQ	174	54%	11%
2004	Tororo	28 days	AS+AQ	194	75%	13%
2004	Arua	28 days	AS+AQ	174	51%	8%
2005	Tororo	28 days	AS+AQ	204	66%	4%
			AL	204	52%	7%
2006	Kampala	28 days	AS+AQ	232	17%	5%
			AL	202	7%	1%
2006	Apac	42 days	AL	210	53%	16%
			DP	211	43%	7%
2007	Kanungu	42 days	AL	199	33%	6%
			DP	215	12%	2%
2008	Tororo	28 days	AL	320	35%	1.0%
			DP	315	11%	0.3%
2012	Apac	28 days	AL	100	31.2%	2.1%
			AS+AQ	100	15.0%	0.0%
2012	Mubende	28 days	AL	102	53.7%	4.3%
			AS+AQ	100	33.6%	0.0%
2012	Kanungu	28 days	AL	100	49.0%	1.1%
			AS+AQ	100	37.4%	0.0%
2016	Arua	42	AL	99	37.4%	0.0%
			DP	98	16.3%	1.1%
2016	Gulu	42	AL	100	65.0%	5.1%
			DP	102	35.3%	1.1%
2016	Mbale	42	AL	101	32.7%	2.3%
			DP	99	23.2%	1.2%
2018	Arua	28	AL	99	39%	8%
		42	DP	96	21%	6%
2018	Apac	28	AL	104	29%	6%
		42	DP	95	13%	2%
2018	Masafu	28	AL	102	59%	13%
		42	DP	94	26%	8%

1.2 Rationale

Routine monitoring of the efficacy of antimalarial drugs is necessary for effective case management and early detection of resistance. Efficacy results can be used as a basis for national treatment policy and for global surveillance of antimalarial drug efficacy. As the parasite evolves continuously to develop resistance to medicines, continuous global monitoring and reporting of drug efficacy and parasite resistance are needed. Only a programme of surveillance of therapeutic efficacy and antimalarial drug resistance will allow detection of changing patterns of parasite susceptibility and timely revision of national (and global) malaria treatment policies. Without regular monitoring and reporting of antimalarial drug resistance, the disease burden and the economic costs of malaria will rise dramatically.

The results of this study will be used to assist the Ministry of Health of Uganda in assessing the current national treatment guidelines for uncomplicated *P. falciparum* malaria and to update the policy if necessary.

1.3 Study drugs

1.3.1 Artemether-Lumefantrine (AL)

AL is a fixed dose-combination (FDC) drug containing the artemisinin derivative, artemether, and lumefantrine. Artemether-lumefantrine (AL) is the first fixed-dose ACT regimen to be manufactured under Good Manufacturing Practice conditions, and is the most widely adopted ACT regimen used in malaria control programs. AL is approved for the treatment of uncomplicated malaria in adults, children and infants, and as treatment of uncomplicated malaria in nonimmune travellers returning from malarious areas. AL is efficacious for treating uncomplicated malaria in children and the frequency of associated adverse events is not higher than other available ACT regimens [26]. AL demonstrates cure rates above 90%, even in areas of multiple drug resistance [27-30]. Post marketing experience based on the delivery of over 250 million treatments has not identified any new safety concerns for AL apart from hypersensitivity and allergies, known class effects of artemisinin derivates [31]. However, the optimal use of AL in the public health system is challenged by the twice daily dosing scheme. Recent anti-malarial resistance monitoring in Angola has shown efficacy of AL in certain sites below the key 90% lower limit of efficacy recommended for artemisinin-based combination therapy [32].

1.3.2 Dihydroartemisinin-piperaquine (DP)

DP is a fixed combination drug containing the artemisinin derivative, dihydroartemisinin, and piperaquine. Dihydroartemisinin is highly active and also the main in vivo metabolite of the drugs artesunate and artemether. Piperaquine is a bisquinoline that retains activity against

chloroquine-resistant *P. falciparum*. Piperaquine replaced chloroquine as the first-line treatment for malaria in China in 1978. DP has proved to be a safe, well tolerated, and highly effective treatment of *P.falciparum* malaria in Asia and Africa with efficacy over 95% [24, 33-36]. DP is as efficacious as AL in treating uncomplicated malaria in African children from different endemicity settings, and shows a comparable safety profile. It has an added advantage because it has a longer post-treatment prophylactic effect [22]. Other studies have similarly shown high efficacy with dihydroartemisinin piperaquine. The drug is well tolerated with no obvious drug associated adverse events [37, 38]. This regimen is relatively inexpensive and has the potential to become an important drug for the treatment of uncomplicated malaria in Africa. In particular, compared to AL it offers decreased cost, simplified dosing, and potentially more reliable drug absorption. More recently, failures of DP have been reported in SE Asia, these are associated with additional parasite polymorphisms in genes and , some have increased plasmepsin copy number. [39, 40]. These findings are very concerning, as they threaten the efficacy of DP to malaria . However, there is as yet no convincing evidence of DP resistance in Africa.

1.3.3 Pyronaridine-artesunate (PA)

Pyronaridine-artesunate, is a fixed dose ACT co-developed by MMV and Shin Poong Pharmaceutical co. Ltd. and is the only ACT to be specifically indicated for the blood-stage treatment of the two dominant strains of malaria: *P. falciparum* and *P. vivax*. The medicine is also available in a child-friendly granule formulation to enhance palatability in this vulnerable population. PA has some advantages over the frequently used AL combination: it does not require fatty food for optimal absorption, it needs to be taken only once per day and the longer half-life (13.2 days for pyronaridine compared to 3.2 days for lumefantrine [7, 8]) may prevent early reinfection. A Cochrane review of the efficacy of PA compared to alternative ACTs for treating people with uncomplicated *P falciparum* malaria showed that PA was efficacious against uncomplicated *P falciparum* malaria, achieving a genotype adjusted treatment failure rate of less than 5% at days 28 and 42, and may be at least as good as, or better than other marketed ACTs [41]. Other studies have demonstrated an equally high treatment efficacy for PA [42-45].

1.3.4. Artesunate - amodiaquine (AS+AQ)

Results from Africa indicate that combinations of AS+AQ are highly effective, although efficacy may be compromised in areas with moderate to high levels of resistance to AQ. The combination of AS+AQ compares well to other treatments and meets the WHO efficacy criteria for use against *Plasmodium falciparum* malaria in many, but not all, the sub-Saharan African countries where it was studied. AS+AQ has now been developed as fixed-dose combination and studies on the fixed dose have shown excellent efficacy and safety [41-43].Concerns have been raised about the possibility of neutropenia in patients following repeated treatment with amodiaquine containing combination however this has not been

demonstrated in any study. Several African countries have chosen AS+AQ as first line treatment.

2.0 OBJECTIVES

The general objective of this study is to assess the therapeutic efficacy and safety of artemether-lumefantrine (AL), pyronaridine-artesunate (PA), artesunate-amodiaquine (ASAQ) and dihydroartemisinin-piperaquine (DP) for the treatment of uncomplicated *P. falciparum* malaria in three sentinel sites in Uganda.

The primary objectives are:

- to measure the clinical and parasitological efficacy of 4 artemisinin-based combination therapies (ACT), artemether-lumefantrine (AL) Pyronaridine-artesunate (PA), artesunate-amodiaquine (ASAQ) and dihydroartemisinin-piperaquine (DP).
- to differentiate recrudescence from new infection of *Plasmodium falciparum* by molecular techniques.

The secondary objectives are:

- to evaluate the incidence of adverse events; and
- to determine the polymorphism of molecular markers mediating resistance for Artemsinin, Pyronaridine, Piperaquine and Lumefantrine resistance.
- to determine the blood concentration of Lumefantrine after treatment with Artemether- Lumefantrine .

3.0 INVESTIGATIONAL PLAN

3.1 Study design

This will be a randomized, open label trial to evaluate clinical and parasitological responses to directly observed treatment for uncomplicated malaria at four sites in Uganda [8, 46]. Children with uncomplicated malaria who meet the study inclusion criteria will be enrolled, treated on site with AL, ASAQ, PA or DP and monitored for 28 days if treated with AL or ASAQ and 42 days if treated with PA or DP. The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or an adequate response. The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drug(s). Molecular analysis of *Plasmodium falciparum* strains will be used to distinguish between a true recrudescence due to treatment failure and episodes of reinfection.

3.2 Study sites

The study will be conducted at Masafu hospital in Busia district, Oli Health Centre IV in Arua district, Kihihi health centre IV in Kanungu district and Moroto hospital in Moroto District. The four health facilities are located in areas with different malaria transmission intensity (table 2).

Table 2. Study sites

District	Health facility	Region type	Transmission intensity
Busia	Masafu Hospital	Rural	High
Arua	Oli HC IV Hospital	Peri Urban	High
Kanungu	Kihihi Health centre	Rural	Moderate
Moroto	Moroto Hospital	Rural	High

Masafu General Hospital is located in Busia District in Eastern Uganda bordering Kenya and serves a population of about 325,000 people. The hospital is situated in an area of high malaria transmission intensity, the prevalence of malaria by microscopy among children aged 0-59 months is at 3% according to the 2019 Malaria Indicator Survey.

Oli Heath Centre IV is located in Arua city, in the West Nile Region. The health Centre is located in an area with a high malaria transmission intensity, the prevalence of malaria by microscopy among children aged 0-69 months was estimated at 22 % according to the 2019 Malaria Indicator Survey.

Kihihi Health centre IV is located in Kihihi Sub County in Kanungu District and serves a population of about 200,000 people. The Health facility is located in an area of moderate malaria transmission with the entomological inoculation rate of 14.2 [47].

Moroto hospital is located in Moroto District in Northern Uganda bordering Kenya and serves a population of about 47,100 people. The health facility is situated in an area of high malaria transmission intensity, the prevalence of malaria by microscopy among children aged 0-59 months is at 34% according to the 2018-19 Malaria Indicator Survey.

All these health centres have inpatient wards where patients can be admitted and all have capacity to manage severe malaria cases.

3.3 Timing and duration of the study

The study will be conducted during the malaria transmission season, from May 2021 to September 2023

3.4 Study population

The study population will consist of children with uncomplicated *P. falciparum* malaria attending the study health clinic who are aged 6 months to 10 years. Parents or guardians will give informed consent on behalf of children (appendix 10, 11,12 and 13).

3.5 Inclusion criteria

- age 6 months to 10 years;
- mono-infection with *P. falciparum* confirmed by positive blood smear;
- parasitaemia of 2000 to 200000 asexual forms/µl;
- presence of axillary temperature ≥ 37.5 °C or history of fever during the past 24 h;
- ability to swallow oral medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule;
- informed consent from parent or guardian;
- Haemoglobin > 8.0 g/dl

3.6 Exclusion criteria

- presence of general danger signs in children aged under 11 years or signs of severe falciparum malaria according to the definitions of WHO (Appendix 1);
- weight under 5 kg;
- mixed or mono-infection with another Plasmodium species detected by microscopy;
- presence of severe malnutrition defined as a very low weight for height (below -3z scores of the median WHO growth standards), by visible severe wasting, or by the presence of nutritional oedema. In a child aged between 6-60 months who has a mid-upper arm circumference < 115 mm;
- presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS);
- regular medication, which may interfere with antimalarial pharmacokinetics (Ongoing prophylaxis with drugs having antimalarial activity such as cotrimoxazole for the prevention of Pneumocystis carini pneumonia in children born to HIV positive women);
- history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s).

3.7 Loss to follow-up

Loss to follow-up occurs when an enrolled patient does not attend the scheduled visits and cannot be found despite all reasonable efforts including following the study participants to the community. No treatment outcome will be assigned to these patients. Every effort must be made to schedule a follow-up visit for patients who fail to return to the study site, especially during but also after administration of the study drug. These patients will be classified as lost to follow-up and censored or excluded from the analysis. Patients who are lost to follow-up but who subsequently return to the study site before the last study day (28 or 42) will not be turned away and will be encouraged to return for check-up visits. The principal investigator will decide whether the patient is to be definitely withdrawn from the study and classified as lost to follow-up on the basis of his or her history and behaviour, or is to be maintained in the study for the final evaluation.

During the follow-up, if infections other than malaria require the administration of medicines with antimalarial activity (appendix 2), the patient will be withdrawn from the study. Patients given tetracycline as eye ointment will not be excluded. Similarly, patients will be withdrawn from the study in case of self-medication or if an antimalarial drug or antibiotics with antimalarial activity is administered by a third party. Patients who are excluded from further study participation after enrollment will still be eligible to receive standard medical care at the study clinic.

3.8 Patient discontinuation or protocol violation

Study patients who meet any of the following criteria will be classified as withdrawn.

- withdrawal of consent. A patient may withdraw consent at any time, without prejudice for further follow-up or treatment at the study site.
- failure to complete treatment, due to:
    - persistent vomiting of the treatment. A patient who vomits the study medication more than twice will be withdrawn from the study and given rescue treatment.
    - failure to attend the scheduled visits on day 1,2,3, and 7; or
    - serious adverse events necessitating termination of treatment before the full course is completed. A patient can be discontinued from the study if the principal investigator decides so due to an adverse event. In this case, information on the adverse event and symptomatic treatment given must be recorded on a case report form. If the adverse event is severe, the principal investigator must notify immediately (latest within 72 hours) and follow the reporting procedures described in section 5.3.
- enrolment violation:
    - severe malaria developed within 24 hours after beginning of the treatment; or
    - erroneous inclusion of a patient who does not meet the inclusion criteria.

- voluntary protocol violation: self- or third-party administration of antimalarial drug (or antibiotics with antimalarial activity) (Appendix 2);
- involuntary protocol violation:
    - occurrence during follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome;
    - detection of mono-infection with another malaria species during follow-up; or
    - misclassification of a patient due to a laboratory error (parasitaemia), leading to administration of rescue treatment.

Patients who are withdrawn will nevertheless be followed up until recovery or the end of follow-up, if possible; however, no treatment outcome will be assigned to these patients, and they will be censored or excluded from the analysis. The reasons for discontinuation or protocol violation will be recorded on the case report form.

4.0 TREATMENT

4.1 Antimalarial treatment

Patients will be randomized to treatment with one of the four study regimens. A minimum of two study drugs shall be assessed at each site. The choice of drugs to be evaluated at each site will be determine by the Ministry of Health National Malaria Control Division. The drug formulation and labelling is summarised in table 3.

Table 3. Drug formulation and labeling

Drug	Trade name (Manufacturer)
Artemether-lumefantrine (20mg/120 mg)	IPCA, India or coartem from Norvatis
1. Pyronaridine-artesunate (Pyramax®) tablet containing 60 mg of artesunate and 180 mg of pyronaridine tetraphosphate 2. Artesunate-pyronaridine (Pyramax®) granules containing 20 mg of artesunate and 60 mg of pyronaridine tetraphosphate.	Pyramax® (Shin Poong Pharmaceutical)
Dihydroartemisinin-piperaquine (40/320mg of dihydroartemisinin (DHA) and piperaquine phosphate (PQ) respectively)	Duo-Cotecxin (Holley-Cotec Pharmaceuticals) or Eurartesim (Sigma-Tau') , or D-artepp (Fosun pharmaceuticals)
Amodiaquine + Artesunate • Infants tablets: AS 25/AQ 67,5 mg • Toddlers tablets: AS 50/AQ 135 mg • Children tablets: AS 100/AQ 270 mg	Winthrop® (Sanofi Aventis)

All subjects will receive the recommended dose of medication for 3 days as below:

- Subjects randomized to the AL group will receive AL twice daily for 3 days, given in fixed dose tablets (20 mg artemether + 120 mg lumefantrine) according to weight-based guidelines. Details of the dosing schedule are included in Appendix 3.
- Subjects randomized to the PA (Pyramax®) will receive PA once daily dose for 3 days according to the patient's body weight. Details of the dosing schedule are included in Appendix 3.
- Subjects randomized to the DP group will receive DP once daily for 3 days given in fixed dose tablets (40 mg dihydroartemisinin + 320 mg piperaquine) according to weight-based guidelines. Details of the dosing schedule are included in Appendix 3.
- Subjects on the ASAQ (Winthrop®) treatment arm will receive AS+AQ daily dose for 3 days according to the patient's body weight (Appendix 3).

4.1.1 Randomization:

Computer generated randomization lists will be created for each of the 4 study sites by a member of the project who will not be directly involved in the conduct of the study. Allocation schedules will be generated using variable blocks and a computer-based random number generator will then be used to select the sequence of blocks and order of treatment group assignment. Sealed copies of the original randomization lists and documentation of the procedure used to generate the lists will be stored at the coordinating offices in Kampala. Prior to the onset of the study, sealed copies of the randomization lists will be distributed to the study nurse responsible for treatment allocation.

4.1.2 Treatment group assignment

Patients will be randomly assigned to one of the four treatment groups (AL, PA, ASAQ or DP). Randomization will be done according to a pre-determined randomization list. The randomization list will be computer generated by a member of the project who will not be directly involved in the conduct of the study. The randomization list will include consecutive treatment numbers with corresponding random treatment assignments.

Table 4. Sample treatment randomization list

Treatment number	Assigned treatment	Study number	Date of treatment assignment	Study drugs administered					
				Day 0		Day 1		Day 2	
				Time	Time	Time	Time	Time	Time
1	AL								
2	PA								
3	DP								
4.	ASAQ								

A separate randomization list will be prepared for each study site. Prior to starting the studies at each site, a project administrator (not involved in the conduct of the study) will print a copy of the treatment randomization list and place it in a sealed opaque envelope to be delivered to the study site. Only the study nurse will have access to the sealed treatment randomization list. At the study sites, treatment allocation and administration of medications will be performed by the study nurse. A sample of the treatment randomization list is included in table 4

4.1.3 Treatment allocation

At the time the first patient is enrolled in the study, the study nurse will break the seal of the envelope containing the treatment randomization list. To allocate subjects to the appropriate treatment group, the study nurse will select the next available treatment number and corresponding study regimen. The study nurse will record the date and time of treatment assignment and the patient's study number. The study nurse will also record when each dose of study medication is given. Only the study nurse will have access to the treatment randomization list and the assigned treatments throughout the conduct of the study. At the completion of the studies at each site the treatment randomization list and assigned treatments will be placed in sealed envelopes and returned to the project administrator not involved in the conduct of the study. Un-blinding of the randomization list will only occur at the time data entry and cleaning are completed for each study.

4.1.4 Administration of study drugs

Administration of all study drugs will be done in the clinic and directly observed. Patients on AL will have the option of remaining at the clinic for their second dose of medication or going home and returning to the clinic later in the day. The study nurse will record the date and time study drugs are administered. The tablets are administered orally with a small amount of still drinking water. For the younger children, the tablets may be dissolved and administered with drinking water. The study nurse will directly observe consumption of study drugs. The study patients will be observed for 30 min after medicine administration for adverse reactions or vomiting. Any patient who vomits the medication within 30 minutes of

administration will be retreated with the same dose and observed for an additional 30 min. If the patient vomits again, he or she will be withdrawn and treated with or referred for treatment with rescue therapy (see section 4.3) as per the national guidelines and recorded as an early treatment failure based on fulfilling criteria for dangers signs.

4.2 Concomitant treatment and medication that should not be used

Fever over 38 °C can be treated with paracetamol or acetaminophen. Parents or guardians will be instructed in the use of tepid sponging, more specifically for children under 5 years of age.

Prior treatment with antimalarial drugs will not be considered an exclusion criterion; however, during follow-up, if infections other than malaria require the administration of medicines with antimalarial activity, the patient will be withdrawn from the study. Patients given tetracycline as an eye ointment will not be excluded (Appendix 2). Patients will be withdrawn from the study in the case of self-medication or if an antimalarial drug or an antibiotic with antimalarial activity is administered by a third party.

Adverse events requiring treatment shall be treated according to the best available local practice. If there is a clinical indication for any additional medication during the course of the study, including medication given to treat an adverse event related to the study medicine, the name of the medicine, the dosage and the date and time of administration must be recorded on the case report form.

The use of herbal remedies during the study should be avoided, and participants should be encouraged to return to the study site for treatment if they feel unwell. If any herbal remedies are taken during the study, this should be captured on the case report form, under ‘study medication administration’.

4.3 Rescue treatment

If a patient vomits more than twice, he or she will receive parenteral therapy with parenteral artesunate (2.4mg/kg/body weight given on admission, at 12 hours and 24 hours then once daily until the patients can take oral ACT) or quinine (10 mg salt/kg body weight 8 hourly until the patients can take oral ACT) according to national treatment guidelines and will be withdrawn from the study.

Any patient with signs of severe or complicated malaria will be hospitalized and will receive parenteral therapy with parenteral artesunate (2.4mg/kg/body weight given on admission, then after 12 hours and 24 hours then once daily until the patients can take oral ACT) or quinine (10 mg salt/kg body weight 8 hourly until the patients can take oral ACT) and relevant supportive treatment according to national treatment guidelines.

If a patient meets one of the criteria for therapeutic failure, he or she will receive quinine tablets, 10mg per kg body weight, given every 8 hours for 7 days according to current national

recommendations. If the patient is reinfected with another malaria species, he or she will receive non study AL or DP according to current national recommendations.

5.0 EVALUATION CRITERIA

The study end-point is the classification assigned to a patient. Valid study end-points include: treatment failure, completion of the follow-up period without treatment failure (adequate clinical and parasitological response), loss to follow-up, withdrawal from study including protocol violation. At all times, the well-being of the patient will take priority over his or her continuation in the study.

5.1 Efficacy and safety evaluation

5.1.1 Classification of treatment outcomes

Treatment outcomes will be classified on the basis of an assessment of the parasitological and clinical outcomes of antimalarial treatment according to the latest WHO guidelines. Thus, all patients will be classified as having early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) or an adequate clinical and parasitological response (ACPR), as defined in Appendix 4.

As parasitological cure is the goal of antimalarial therapy, all study patients who show treatment failure will be given rescue treatment. Follow-up will continue until recovery. The outcome of the rescue treatment in these patients does not need to be recorded systematically for the purpose of the surveillance study.

5.1.2 Safety end-points

The incidence of any adverse event will be documented. All patients will be asked routinely about previous symptoms and about symptoms that have emerged since the previous follow-up visit. When clinically indicated, patients will be evaluated and treated appropriately. All adverse events, drug-related or not, will be recorded on the case report form. Serious adverse events (see definitions in 5.3) must be reported to the sponsor.

5.2 Clinical evaluation

All patients will be evaluated clinically as described below.

5.2.1 Physical examination

A standard physical examination will be performed at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28. Additional physical examination will be performed for study participants on PA and DP on day 35 and 42. A complete medical history, including prior and concomitant medication, demographic information and contact details will be recorded at baseline.

The circumference of the left mid-upper arm will be measured, at the mid-point between the elbow and the shoulder, and will be recorded to the nearest 0.1 cm.

5.2.2 Body weight

Body weight will be recorded on day 0 to the nearest kilogram on a Salter scale or on a hanging scale for young children. The scales will be properly calibrated. The reliability of the scales will be verified before the study begins and checked at regular intervals. Patients should not wear excessive clothing while being weighed as this can overestimate their true weight. All young children should only wear undergarments while being weighed. The screening weight will be used to satisfy the inclusion or exclusion for nutrition status as well as to calculate the dose (number of tablets) to be administered.

5.2.3 Body temperature

Tympanic or axillary, temperature will be measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, 35 and 42. Temperature will be measured with a thermometer that has a precision of 0.1 °C. Temperature will also be measured as clinically indicated. If the result is < 36.0 °C, the measurement will be repeated. The same route should be used throughout the study.

The quality of the temperature-taking technique and the thermometers should be assessed regularly. Thermometers should be tested in a water-bath of known temperature before the study begins and at regular intervals thereafter.

5.2.4 Microscopic blood examination

Thick and thin blood films for parasite counts should be obtained and examined at screening on day 0 to confirm adherence to the inclusion and exclusion criteria. Thick blood films will be examined once on day 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42 or on any other day if the patient returns spontaneously and parasitological reassessment is required. Specimens will be labelled anonymously. The screening number or the patient study number, the date and the day of follow-up will be recorded either on the frosted edge of the slide or on the glass with a permanent glass pen.

A fresh Giemsa stain dilution will be prepared at least once a day and possibly more often, depending on the number of slides to be processed. Giemsa-stained thick and thin blood films will be examined at a magnification of 1000 to identify the parasite species and to determine the parasite density.

Screening smears will be stained rapidly (10% Giemsa for 10–15 min) for initial screening. Slides for enrolled patients and for all follow up visits will be stained more carefully with 2–3% Giemsa for 45–60 minutes. The study number of the patient, the date and the day of follow-up will be recorded on the frosted edge of the slide or on the glass with a permanent glass pen.

The thick blood smear for initial screening will be used to count the numbers of asexual parasites and white blood cells in a limited number of microscopic fields. The adequate parasitaemia for enrolment is at least one parasite for every three white blood cells,

corresponding to approximately 2000 asexual parasites per microlitre, for high transmission areas.

The smear obtained after enrolment will be used to calculate the parasite density, by counting the number of asexual parasites in a set number of white blood cells (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of white blood cells will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 white blood cells have been reached, the count will be stopped after the reading of the last field has been completed. Parasite density, expressed as the number of asexual parasites per µl of blood, will be calculated by dividing the number of asexual parasites by the number of white blood cells counted and then multiplying by an assumed white blood cell density (typically 6000 per µl).

$$\text{Parasite density (per µl)} = \text{number of parasites counted} \times \frac{(8000)}{\text{Number of leukocytes counted}}$$

The same technique will be used to establish the parasite count on each subsequent blood film. When the number of asexual parasites is less than 100 per 200 white blood cells in follow-up smears, counting will be done against at least 500 white blood cells (i.e. to completion of the field in which the 500th white blood cell is counted).

A blood slide will be considered negative when examination of 1000 white blood cells or 100 fields containing at least 10 white blood cells per field reveals no asexual parasites. The presence of gametocytes on an enrolment or follow-up slide will be recorded, but this information will not contribute to basic evaluation.

In addition, 100 fields of the second thick film at day 0 will be examined to exclude mixed infections; in case of any doubt, the thin film will be examined for confirmation. If examination of the thin film is not conclusive, the patient will be excluded from the analysis after complete treatment and follow-up.

To detect the presence of gametocyte, at least 1000 white blood cells should be counted.

Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (differences between the two microscopists in species diagnosis, in parasite density of > 50% or in the presence of parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

5.2.5 Genotyping of malaria parasites

In order to differentiate a recrudescence (same parasite strain) from a newly acquired infection (different parasite strain), a genotype analysis will be conducted. This is based on the extensive genetic diversity among the malaria parasite genes. The genotypic profiles of pre- and post-parasite strains are compared.

In order to minimize discomfort to the patient due to repeated finger pricks, 3-4 drops of blood will be collected on filter paper (whatman filter paper grade 3 or whatman 903 protein-saver cards) during screening or enrolment and each time blood smears are required according to the protocol on and after day 7. Specimens will be labelled anonymously (patient study number, date and day of follow-up), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analysed. When these conditions cannot be achieved, for example in extremely humid environments where air-conditioning is not available, storage in a refrigerator or freezer may be considered, but great care must be taken to protect samples from frost and moisture. Parasite DNA will subsequently be removed from the filter paper and prepared for molecular analysis using a chelex or qiagen extraction method. Genotyping will be performed on all patients with outcomes classified as LCF or LPF on day 7 or after. Genotyping of parasites collected at baseline (day 0) and the day of recurrent parasitemia will be done to distinguish between true recrudescence and new infections.

Briefly, selected regions of the msp1, msp2, glurp or microsatellites will be amplified using PCR and characterized based on sequence and size polymorphisms identified by capillary electrophoresis. Genotyping patterns on the day of recurrent parasitemia will be compared with those at treatment initiation. The posterior probability for recrudescence for all LPF and LCF will be summed to derive the total number of recrudescence in efficacy calculations. For tabulation, a posterior probability of above 50% will be considered a recrudescence. [48]. Additional molecular studies will include analyses of polymorphisms in parasite genes for mutations that may impact on malaria infection and response to antimalarial therapy. The additional samples from study participants who consented for future use of the biological samples will be stored at Molecular Laboratory in Mulago.

5.2.6 Molecular markers for antimalarial drug resistance

Two to three drops of blood will be collected on whatman filter paper grade 3 or whatman 903 protein-saver cards, on day 0 and day of failure to study the single nucleotide polymorphisms or copy number of *K*13, *plasmepsin*, and *pfmdr1*, which are considered as markers of resistance to artemisinin, and their partner drugs. Specimens will be labelled anonymously (patient study number, day of follow-up, date), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analysed. DNA extracted from DBS will be used to analyse molecular markers mediating antimalarial resistance.

5.2.7 Hemoglobin measurement

Hemoglobin will be measured from fingerprick blood samples using a portable spectrophotometer (HemoCue, Anglom, Sweden). Hemoglobin will be measured on Day 0 and at the time of clinical treatment failure (ETF or LCF). Haemoglobin will be systematically measured in all patients from Day 21 onwards.

5.2.8. Pharmacokinetics lab procedures

Blood samples will be collected on day seven to determine plasma concentrations of lumefantrine. Plasma concentrations will be determined from blood samples (200 ul-1 ml) obtained from all the study participants on the AL treatment arm by finger-prick or phlebotomy. Blood samples will be centrifuged and plasma immediately frozen. Drug concentrations will be determined using liquid chromatography with tandem mass spectrometry or related methods at the UCSF Drug Research Unit [49-51]. Specimens will be labelled anonymously with the patient study number and date.

5.3 Safety assessment

Safety will be assessed by recording the nature and incidence of adverse events and serious adverse events. Adverse events will be assessed by direct questioning. An adverse event is defined as any unfavourable, unintended sign, symptom, syndrome or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product. All adverse events must be recorded on the case report form.

A serious adverse event is defined as an experience that results in any of the following outcomes:

- death,
- life threatening condition;
- hospitalization or prolongation of hospitalization;
- persistent or significant disability or incapacity.

'Life-threatening' means that the person was at immediate risk for death; it does not refer to an adverse event that might have caused death if it were more severe. 'Persistent or significant disability or incapacity' means that a person's ability to carry out normal life functions is substantially disrupted.

5.3.1 Identification and reporting of adverse events

At each follow-up visit (days 1, 2, 3, 7, 14, 21, 28, 35, 42 and any unscheduled day), study clinicians will assess patients according to a standardized clinical record form. A severity grading scale, based on toxicity grading scales developed by the WHO and the National Institutes of Health, Division of Microbiology and Infectious Diseases, will be used to grade severity of all symptoms, physical exam findings, and hemoglobin results (Appendix 11). Any new event, or an event present at baseline that is increasing in severity, will be considered an adverse event.

For each possible adverse event identified and graded as moderate, severe or life threatening, an adverse event report form will be completed. An adverse event report form will not be completed for events classified as mild as these symptoms are common and difficult to distinguish from signs and symptoms due to malaria. The following information will be recorded for all adverse experiences that are reported:

- Description of event
- Date of event onset
- Date event reported
- Maximum severity of the event
- Maximum suspected relationship of the event to study medication
- Is the event serious?
- Initials of the person reporting the event
- Was the event episodic or intermittent in nature?
- Outcome
- Date event resolved

5.3.2 Reporting of serious adverse events

Guidelines for reporting of serious adverse events provided by Makerere University School of Biomedical Research (SBSREC) IRB, and the Ugandan National Council for Science and Technology (UNCST) and the National Drug Authority (NDA) will be followed. When a serious adverse event (SAE) occurs, the IRBs shall be notified. All serious events shall be reported to the IRB's within the prescribed timelines as shown in table 4.

Table 5. Timelines for reporting adverse events

Institution	Type of Adverse Events	When to Report
NDA	1. All Serious* or Unexpected± events irrespective of relationship	• Death and Life-threatening events within 48-hours by phone, fax or email with report submitted within 7-calendar days • All other reportable events within 15-calendar days of awareness
UNCST	2. All Serious* or Unexpected± events irrespective of relationship	• Death and Life-threatening events within 48-hours by phone, fax or email with report submitted within 7-calendar days • All other reportable events within 15-calendar days of awareness
SBSREC	3. All Serious* or Unexpected± events irrespective of relationship	• Fatal or life-threatening events within 3 working days of awareness • All other SAEs within 10 working days of awareness

5.3.3 Management of serious adverse events

For all SAEs, the relationship of the events to the study medication shall be determined by the clinicians. If a patient experiences an SAE while receiving treatment for uncomplicated malaria with their assigned study treatment, the study medication will be stopped if the SAE

is felt to be probably or definitely related to the study medication and the patient will be treated with quinine. Plans for additional follow-up, including laboratory tests, will be individualized to each subject as appropriate for the clinical setting. If an SAE occurs while a patient is not currently receiving treatment with their study medications, treatment and plans for follow up will be individualized to each subject as appropriate for the clinical setting.

6.0 STUDY ASSESSMENT

6.1 Screening and enrolment

All patients who meet the basic enrolment criteria (age, fever or history of fever if appropriate, symptoms of malaria, absence of danger signs in children in relation to malaria - child unable to drink or breastfeed, vomiting everything, recent history of convulsions, lethargic or unconscious state, unable to sit or stand, difficulty in breathing, absence of signs of severe malaria, absence of severe malnutrition) during screening will be assigned a consecutive number and evaluated in greater depth by clinical staff. Care will be taken to detect early signs of febrile diseases other than malaria, as their presence will necessitate exclusion from the evaluation. The most frequent confounding condition is a lower respiratory tract infection: cough or difficult breathing, together with fast breathing, is an indicator for exclusion. Fast breathing is defined as a respiratory frequency > 50/min in infants under 12 months of age, > 40/min in children aged 12–59 months and chest indrawing in older children. Other relatively common febrile conditions are otitis media, tonsillitis, measles and abscesses. Patients with these conditions will not be enrolled but should be treated for both malaria (if they have parasitaemia) and the other infection, as appropriate. Patients with severe conditions will be referred immediately to an appropriate health facility for further care.

The screening record form (Appendix 5) will be used to record the general information and the clinical observations on each patient being screened. If the patient meets the clinical criteria, he or she will be examined for parasitaemia. Once the patient meets all the enrolment criteria, a parent or guardian will be asked for consent to participate in the study.

6.2 Follow-up

Patients who meet all the enrolment criteria will be given a personal identification number (patient study number) and will receive treatment only after the study has been fully explained to them and they have willingly provided informed consent. Any person who decides not to participate in the study will be examined, treated and followed-up by the health facility staff according to the standard of care established by the Ministry of Health.

The basic follow-up schedule and activities are summarized in table 6, Appendix 6). A case report form (Appendix 7) and a serious adverse event report form (Appendix 8) will be used to record the general information and clinical observations on each patient enrolled into the study. The appointment schedule will be clearly explained, and a follow-up card with the patient study number will be provided.

Table 6. Follow-up Schedule

Days	D0		D1		D2		D 3	D 7	D 14	D 21	D 28	D35	D 42	D ..
Hours	**0**	**8**	**24**	**36**	**48**	**60**								
Study drugs	X	X	X	X	X	X								
History	X						X	X	X	X	X	X	X	X
Temperature	X						X	X	X	X	X	X	X	X
Physical exam	X						X	X	X	X	X	X	X	X
Thick blood smear	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thin blood smear	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Filter paper sample	X							X	X	X	X	X	X	X
Hemoglobin	X						†	†	†	X	X	X	X	†
Plasma sample (AL arm)								X						
Assessment for AEs	X		X		X		X	X	X	X	X		X	X

X = perform this task

† Performed only if clinical treatment failure

The day a patient is enrolled and receives the first dose of medicine is designated ‘day 0’. All antimalarial treatment will be given by a study team member under supervision. Enrolled patients will be observed for at least 30 min after treatment to ensure that they do not vomit the medicine. If vomiting occurs within 30 min of treatment, the full treatment dose will be repeated. Ancillary treatment, such as antipyretics, will be provided if necessary to patients by the study team and documented on the case report form. Patients with persistent vomiting (i.e. necessitating more than a single repeat dose) will be excluded from the study and immediately referred to the health facility staff for appropriate management.

Thereafter, patients are required to undergo regular clinical reassessment. Blood films for parasite counts will be made daily on days 1, 2, 3, 7, 14, 21, 28, 35 and 42. Parents or guardians will be advised to return on any day during the follow-up period if symptoms return and not to wait for the next scheduled visit day. In particular, parents or guardians should be instructed to bring children to the centre at any time if they show any sign of danger (unable to drink or breastfeed, vomiting everything, presenting with convulsions, lethargic or unconscious, unable to sit or stand, presenting with difficult breathing), if they are still sick or if there is any cause for worry. Clinical reassessment will be sufficiently thorough to ensure patient safety and will include assessment not only for potential treatment failure but also

for potential adverse reactions to the medicine. Additionally, blood films will be obtained whenever parasitological reassessment is requested by the clinical staff.

Because many medicines have to be given over several days, the initial visits are critical not only for assessing efficacy but also for ensuring patient safety; defaulters at this stage will not have received a complete course of treatment and may be at risk for clinical deterioration. All reasonable efforts will be made to find defaulters to ensure complete treatment. Similarly, the ultimate success of the study rests on minimizing loss to follow-up. While patients are encouraged to return on their own for scheduled follow-up visits, provisions will be made ahead of time for locating patients at home if they do not attend as requested. Detailed directions to the home of the participants shall be obtained during enrolment, and study team members familiar with the community will be responsible for home visits and means of transport for patients follow-up will be provided.

The schedule of treatment and follow-up examinations given in this protocol must be followed to ensure data integrity. Patients who fail to return on days 1 and 2 and miss one dose of the treatment will be withdrawn from the study definitively. After day 3, patients who fail to return on day 7 but are present on day 6 or 8 (likewise days 13/15, days 20/22, days 27/29, days 34/36 and days 41/43) may still be included in the analysis. Deviation from the protocol of more than 1 day should, however, be avoided.

7.0 DATA COLLECTION AND MANAGEMENT

The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the case report form. Laboratory and clinical data will be recorded on a daily basis on the case report form designed for the study. Data derived from source documents should be consistent with the source documents, or the discrepancies should be explained. Any change or correction to a case report form should be dated and explained and should not obscure the original entry. All case report forms will be checked for completeness.

After the study has been completed, data will be entered into a database by double independent data entry, according to WHO standard procedures. [52]The study data will be stored in a computer database, maintaining confidentiality. Case record forms will be provided for each subject. Participants will be identified by their initials and study identification number on the case record form. Patient names will not be entered into the computerized database. All patient record forms will be kept in individual files in a secure filing cabinet in the study clinic. All corrections will be made on case record forms by striking through the incorrect entry with a single line and entering the correct information adjacent to it. The correction will be initialed and dated by the investigator. Any requested information that is not obtained as specified in the protocol will have an explanation noted on the case record form as to why the required information was not obtained. Additional records will be kept in the clinical and laboratory record books at the core facility in Kampala. The investigators will allow all requested monitoring visits, audits or reviews.

The principal investigator is responsible for keeping all screening forms, case report forms and the completed subject identification code list in a secure location (patient screening and enrolment logbook).

All members of the study team will be trained on the study protocol prior to the onset of the trial. The study clinicians will complete case record forms at each patient visit. These forms will be reviewed by the study coordinator and site supervisors for completeness and accuracy. For quality control of thick blood smear slide readings, expert microscopists who will be blinded to the patient's treatment group will repeat the reading of all slides. All discrepant slide readings will be resolved based on the results of a 3rd reading. Study group meetings will be conducted by the coordinator once a week to assess progress of the study, address any difficulties, and provide performance feedback to the members of the study group. In addition, members from the core facility will make regular visits to active study sites as needed. Study monitoring shall be done by monitors from IDRC, Ministry of health and by reciprocal monitoring from the WHO.

8.0 STATISTICAL METHODS

8.1 Minimum sample size

The sample size calculations will be based on estimated failure rate for the different drugs. For the AL treatment arm, we estimate that the failure rate will not exceed 10%, with a 5% margin of error and 95% Confidence Interval. The same size will be adjusted by 40% to account for the high rate of new infection rate among those receiving treatment with AL thus the total sample size of approximately 200. For DP, PA and ASAQ treatment arm, the failure rate is estimated at 5% with a 5% margin of error and 95% Confidence interval. The same size will also be adjusted by 35% to account for the new infection rate thus the total sample size of approximately 100

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8.2 Analysis of data

Data analysis will be primarily performed using WHO Excel software designed for analyzing therapeutic efficacy as well as STATA statistical software packages. Nonlinear mixed effects modeling in NONMEM will be used for PK data. In addition to the reasons for withdrawal listed in section 3.8, patients will be censored or excluded from the analysis if the PCR results are unclassifiable or if the results of PCR indicate that the failure is due to reinfection with P. falciparum or P. vivax.

Descriptive statistics will be used to summarize baseline characteristics of study patients. Efficacy and safety data will be evaluated using a modified intention-to-treat analysis and will only include patients who meet all selection criteria. Categorical variables will be compared between the treatment groups using chi-square tests or Fisher's exact tests and continuous variables will be compared using t-tests or non-parametric tests. A p-value of < 0.05 will be

considered statistically significant. Estimates of the risk of failure for all primary outcomes will be made using the Kaplan-Meier product limit formula.

The final analysis will include:

- a description of all patients screened and the distribution of reasons for non-inclusion in the study;
- a description of all the patients included in the study;
- the proportion of adverse events and serious adverse events in all the patients included in the study;
- the proportion of patients lost to follow-up or withdrawn, with 95% confidence intervals and a list of reasons for withdrawal;
- the cumulative incidence of success and failure rates at day 28/42, PCR-uncorrected and PCR-corrected; and
- The proportion of early treatment failure, late clinical failure, late parasitological failure and adequate clinical and parasitological response at day 28/42, with 95% confidence intervals, PCR-uncorrected and PCR-corrected.

8.2.1 Primary outcomes

Primary outcomes will be based on the risk of parasitological treatment failure (all ETF, LCF and LPF) after 28 or 42 days of follow-up unadjusted and adjusted by genotyping to distinguish recrudescence from new infections. Risks of treatment failure will be estimated using the Kaplan-Meier product limit formula based on a modified intention-to-treat analysis.

8.2.2 Secondary outcomes

- Prevalence of fever (defined as both subjective fever in the previous 24 hours and measured axillary temperature $\geq$ 37.5°C) on follow-up days 1, 2, and 3.
- Prevalence of parasitemia on follow-up days 1, 2 and 3.
- Change in mean hemoglobin from day 0 to day 28 or 42 (or day of rescue therapy for patients classified as LCF or LPF).
- Prevalence of gametocytemia and gametocyte density on follow-up days 1, 2, 3, 7, 14, 21, 28, 35 and 42.
- Risk of serious adverse events: proportion of patients experiencing any serious adverse event in each treatment group during the 42-day follow-up period (both including and excluding patients classified as ETF or LCF, as recurrent malaria can be confounding).
- Risk of adverse events of moderate or greater severity, at least possibly related to the study medications (both including and excluding patients classified as ETF or LCF).
- Prevalence of molecular markers associate with drug resistance from day 0 to the day of recurrent parasitemia (prevalence of *P. falciparum pfcrt* and *pfmdr1*, K13, and plasmepsin polymorphisms associated with altered drug sensitivity). Association

between antimalarial drug concentration and treatment failure or detection of resistant polymorphisms.

We shall use the WHO definitions of efficacy outcome as defined below:

Early treatment failure (ETF)

- Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia
- Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature
- Parasitaemia on day 3 with axillary temperature ≥ 37.5°C
- Parasitaemia on day 3 ≥ 25% of count on day 0

Late clinical failure (LCF)

- Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and last day of follow up in patients who did not previously meet any of the criteria of early treatment failure
- Presence of parasitaemia on any day between day 4 and last day of follow up with axillary temperature ≥ 37.5°C in patients who did not previously meet any of the criteria of early treatment failure

Late parasitological failure (LPF)

I. Presence of parasitaemia on any day between day 7 and last day of follow up with axillary temperature < 37.5°C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure

Adequate clinical and parasitological response (ACPR)

II. Absence of parasitaemia on the last day of follow up , irrespective of axillary temperature, in patients who did not previously meet any of the criteria

Guidelines on calculating the cumulative success or failure rate, the proportion of adequate clinical and parasitological response and treatment failure are given in Appendix 9.

8.3 Dissemination of results

At the end of the study, the principal investigator will submit a report on the study and its main outcome. This report will be shared with the National Malaria Control Programme, Ministry of Health and will allow to formulate recommendations and to enable the Ministry of Health to make informed decisions about whether the current national antimalarial treatment guidelines should be updated. The patient data will be shared with WHO and WWARN for inclusion into the WHO global database.

The study will be presented during a scientific meeting and will be published in scientific journals.

Each patient will be informed about her/his treatment outcome on the last day of follow-up visit. Furthermore, the results of the study will be shared with relevant local health authorities (study health facilities, Ministry of health etc.). The primary goal is to support the ministry of Health National Malarial Control Division to make informed decision on the treatment guideline for malaria and the secondary goal is to publish the findings of the study.

8.4 Amendments to the protocol

After the protocol has been accepted, no change may be made without the agreement of the principal investigator, the sponsor(s) and the institutional review boards.

9.0 ETHICAL CONSIDERATIONS

9.1 Approval by the national ethical committee

Before the study, official approval to conduct the study will be obtained Makerere University School of Biomedical Sciences IRB, the Ugandan National Council of Science and Technology and the National drug Authority. Any amendments or modifications to this material will be reviewed and approved by these bodies prior to implementation. Before commencement of patient enrolment, the study key information will be posted on clinical trial registry (https://clinicaltrials.gov/) or other related registries.

9.2 Informed consent

Patients will be included in the study only if parents or guardians of children give informed consent. The consent request, available in English and translated into local languages (Luganda, Runyakitara, Lugbara, Samia, Kiswahili), will be read entirely to parent or guardian. Children aged 8 to 10 years will provide assent in addition to the parental consent to participate in the study. Details about the study and its benefits and potential risks will be explained. Once any questions have been answered, a signature will be requested on the document (Appendix 10 and 11). If parent or guardian is illiterate, a literate witness must sign; if possible, the signatory will be selected by the prospective participant and will have no connection to the research team.

9.3 Confidentiality

All information on patients will remain confidential and be shared only by the study team. Unique identifiers will be used for computer-based data entry and blood samples. In all cases, the principal investigator will ensure that all screening forms, the case report forms and the completed subject identification code list (patient screening and enrolment logbook) are kept in locked files.

9.4 Health-care services

Free health care throughout follow-up for any illness related to malaria will be provided to the study patients regardless of treatment outcome; this includes any expenses related to hospital admission and to adverse medicine reactions, if required.

When prospective or actual participants are found to have diseases unrelated to malaria, the principal investigator should advise parents or guardians to obtain, or refer them for, medical care.

Any patient who decides not to participate or who cannot be enrolled into the study because he or she does not meet all inclusion criteria will not be enrolled but will be referred to the health facility staff and will be treated for malaria (if they have parasitaemia) with the first line treatment and/or other diseases. Such people will be treated and followed-up according to the standard of care established by the Ministry of Health. The principal investigator will ensure that this antimalarial drug is available at the health centre. Patients with severe conditions will be referred to the nearest health facility where they can receive appropriate care.

If a patient is withdrawn from the study before he or she has completed the full course of the treatment, the physician must make all necessary arrangements to provide the patient with the full dose of the medicine being tested or with a full course of the first-line treatment recommended in the national policy.

9.5 Reimbursement and compensation

Subjects shall be reimbursed for their transport to attend all visits to the health centre. No other gifts or payments will be made.

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APPENDIX 1. DEFINITION OF SEVERE FALCIPARUM MALARIA

Severe manifestation of *P. falciparum* malaria in adults and children[53]

Clinical manifestations

- prostration;
- impaired consciousness;
- respiratory distress (metabolic acidosis);
- multiple convulsions;
- circulatory collapse;
- pulmonary oedema (radiological);
- abnormal bleeding;
- jaundice;
- haemoglobinurea.

Laboratory findings

- severe anaemia (haemoglobin < 5 g/dl, haematocrit < 15%);
- hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dl);
- acidosis (plasma bicarbonate < 15 mmol/l);
- hyperlactataemia (venous lactic acid > 5 mmol/l);
- hyperparasitaemia (> 4% in non-immune patients);
- renal impairment (serum creatinine above normal range for age).

Classification of severe malaria in children

Group 1: children at increased risk for death

- prostration;
- respiratory distress.

Group 2: children at risk for clinical deterioration

- haemoglobin < 5 g/dl, haematocrit < 15%;
- two or more convulsions within 24 h.

Group 3: children with persistent vomiting

Children who require parenteral treatment because of persistent vomiting but who lack any specific clinical or laboratory features of groups 1 or 2 above.

APPENDIX 2. MEDICATIONS (WITH ANTIMALARIAL ACTIVITY) THAT SHOULD NOT BE USED DURING THE STUDY PERIOD IN ADDITION TO THE STUDY DRUG(S)

- chloroquine, amodiaquine;
- quinine, quinidine;
- mefloquine, halofantrine, lumefantrine;
- artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin);
- proguanil, chlorproguanil, pyrimethamine;
- sulfadoxine, sulfalene, sulfamethoxazole, dapsone;
- primaquine (for *P. vivax*)
- atovaquone;
- antibiotics: tetracycline*, doxycycline, erythromycin, azythromycin, clindamycin, rifampicin, trimethoprim;
- pentamidine.

APPENDIX 3. DOSING CHART OF ARTEMETHER-LUMEFANTRINE, PYRONARIDINE-ARTESUNATE AND DIHYDROARTEMISININ -PIPERAQUINE

Artemether-lumefantrine (20mg/120mg)

Body weight (kg)	Number of tablets					
	Day 0		Day 1		Day 2	
	0 hrs	8 hrs	24hrs Morning	36hrs Evening	48hrs Morning	60hrs Evening
5 – <14	1	1	1	1	1	1
15 – 24	2	2	2	2	2	2
25 to <35	3	3	3	3	3	3
35 kg and above	4	4	4	4	4	4

Pyronaridine artesunate

Body weight ranges (kg)	PA dose	Number of tablets		
		Day 0	Day 1	Day 2
15 - < 24 kg	Tablets containing 60 mg of artesunate and 180 mg of pyronaridine tetraphosphate	1	1	1
24 - <45 kg	Tablets containing 60 mg of artesunate and 180 mg of pyronaridine tetraphosphate	2	2	2
		Sackets of granules		
		Day 0	Day 1	Day 2
5 - < 8 kg	Granules containing 20 mg of artesunate and 60 mg of pyronaridine tetraphosphate.	1	1	1
8 - < 15 kg	Granules containing 20 mg of artesunate and 60 mg of pyronaridine tetraphosphate.	2	2	2
15 - < 20	Granules containing 20 mg of artesunate and 60 mg of pyronaridine tetraphosphate.	3	3	3
20 - < 25	Granules containing 20 mg of artesunate and 60 mg of pyronaridine tetraphosphate.	4	4	4

Dihydroartemisinin+piperaquine (40/320 mg)

Body weight (kg)	DP dose (mg)	Day 0	Day 1	Day 2
5 to < 8	20+160	½ tablet	½ tablet	½ tablet
8 to < 11	30+240	¾ tablet	¾ tablet	¾ tablet
11 to < 17	40+320	1 tablet	1 tablet	1 tablet
17 to < 25	60+480	1 ½ tablets	1 ½ tablets	1 ½ tablets
25 to < 36	80+640	2 tablets	2 tablets	2 tablets

Artesunate-amodiaquine

Body weight ranges (kg)	ASAQ dose	Number of tablets		
		Day 0	Day 1	Day 2

≥4.5 to < 9	25mg Artesunate/67.5 mg Amodiaquine	1	1	1
9 to <18	50mg Artesunate/135 mg Amodiaquine	1	1	1
18 to <36	50mg Artesunate/135 mg Amodiaquine	2	2	2

APPENDIX 4. CLASSIFICATION OF TREATMENT OUTCOMES[1]

Early treatment failure

- danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature ≥ 37.5 ºC;
- parasitaemia on day 3 ≥ 25% of count on day 0.

Late treatment failure

Late clinical failure

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 42 in patients who did not previously meet any of the criteria of early treatment failure;
- presence of parasitaemia on any day between day 4 and day 42 with axillary temperature
  ≥ 37.5 ºC or history of fever in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure

- presence of parasitaemia on any day between day 7 and day 42 with axillary temperature
  < 37.5 ºC in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response

- absence of parasitaemia on day 42, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

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APPENDIX 5. CASE SCREENING FORM

Case screening form	
Health centre name: Locality: District:	Study number: Patient screening number: Date of visit: dd/mmm/yyyy
Demographic data	
Date of birth: dd/mmm/yyyy Height (cm): Weight (kg):	or estimated age: in: ☐ months or ☐ years Sex: ☐ Male ☐ Female
Pre-treatment temperature	
History of fever in previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral	
Blood analysis	
<u>Parasitology</u> Approximate number of *P. falciparum* asexual parasites: Presence of 1 parasites/3 white blood cells? ☐ Yes ☐ No **(If no, patient is not eligible)** Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible)** If yes which species: ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has blood sample for PCR been collected? ☐ Yes ☐ No	
<u>Haematology</u> Haemoglobin: g/dl	
Inclusion criteria	
• age 6 months to 10 years; • mono-infection with *P. falciparum* confirmed by positive blood smear; • parasitaemia of 2000 to 200000 asexual forms/µl; • presence of axillary temperature ≥ 37.5 °C or history of fever during the past 24 h; • ability to swallow oral medication; • ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; • informed consent from parent or guardian;	
Does the patient meet all the inclusion criteria? ☐ Yes ☐ No **(If no, patient is not eligible)**	
Exclusion criteria	
• presence of general danger signs in children aged under 12 years or signs of severe falciparum malaria according to the definitions of WHO; • weight under 5 kg; • mixed or mono-infection with another *Plasmodium* species detected by microscopy; • presence of severe malnutrition defined as a child aged between 6-60 months who has a mid-upper arm circumference < 115 mm; • presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS); • regular medication, which may interfere with antimalarial pharmacokinetics (Ongoing prophylaxis with drugs having antimalarial activity such as cotrimoxazole for the prevention of Pneumocysti carini pneumonia in children born to HIV positive women); • history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or	

used as alternative treatment(s).
Does the patient meet any of the exclusion criteria? ☐ Yes ☐ No **(If yes, the patient is not eligible)**
If yes, please specify the reason for exclusion:

Patient informed consent and assent	
Consent form signed: ☐ Yes ☐ No **(If no, the patient is not eligible)** Assent form signed: ☐ Yes ☐ No **(If no, the patient is not eligible)**	Patient study number: Date: dd/mmm/yyyy

APPENDIX 6. FOLLOW-UP ACTIVITIES

Day 0

Screening

- clinical assessment, including measurement of weight and height; referral in cases of severe malaria or danger signs;
- measurement of temperature;
- parasitological assessment;
- informed consent

Enrolment

- treatment;
- blood sampling for genotyping.

Optional

- haemoglobin;
- molecular markers of drug resistance;

Day 1

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of temperature;
- parasitological assessment in cases of severe malaria or danger signs or if parasite clearance needs to be calculated;
- treatment or alternative treatment in case of severe malaria.

Day 2

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- treatment or alternative treatment in case of early treatment failure.

Day 3, day 7, day 14, day 21, day 28, day 35, day 42 or any other day

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- alternative treatment in cases of treatment failure;
- blood sampling for genotyping to distinguish between recrudescence and reinfection in cases of treatment failure after day 7.
- haemoglobin/haematocrit;
- Blood sampling for measuring plasma level of Lumefantrine on day 7
- blood sampling for molecular markers for drug resistance

APPENDIX 7. CASE REPORT FORMS

Case report form: follow-up day 0	
Health centre name: Locality: District:	Study number: Patient study number: Date of visit: dd/mmm/yyyy
Demographic data	
Date of birth: dd/mmm/yyyy Height (cm): Weight (kg):	or estimated age: in: ☐ months or ☐ years Height (cm):
Pre-treatment temperature	
History of fever in previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral	
Thick blood smears for *P. falciparum*: quantitative parasite counts and qualitative gametocyte counts	
Average number of asexual *P. falciparum* parasites/□l: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible)** If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has blood sample for PCR been collected? ☐ Yes ☐ No	
Prior medication	
All prior medication, including natural remedies and homeopathic medicines, taken within the previous 14 days should be reported in this section. Has the patient taken any prior antimalarial medication? ☐ Yes ☐ No. If yes, please specify below. Either the date of stopping or the 'ongoing' box should be checked.	

Medicine name (generic name)	Dates	Ongoing (Yes = ☒)	Total daily dose and unit	Route of administration	Indication for use
	Start: dd/mm/yyyy Stop: dd/mm/yyyy	☐			
	Start: dd/mm/yyyy	☐			

	Stop: dd/mm/yyyy				

Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 1
Study number:
Patient study number:
Date of visit : dd/mm/yyyy
Clinical status
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No
If yes, perform thick blood smear
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral
Thick blood smears for estimation of *P. falciparum* parasite counts
Average number of asexual *P. falciparum* parasites/µl:
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No
Were species other than *P. falciparum* present? ☐ Yes ☐ No
If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*
Adverse events
Presence of an adverse event? ☐ Yes ☐ No
If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 2				
Study number: Patient study number: Date of visit : dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/□l: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 3				
Study number: Patient study number: Date of visit : dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 7				
Study number: Patient study number: Date of visit : dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No Has a blood sample for plasma level of lumefantrine been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: follow-up day 14

Study number:

Patient study number:

Date of visit : dd/mm/yyyy

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 24 h? ☐ Yes ☐ No

Temperature: ºC ☐ Axillary

Thick blood smears for estimation of *P. falciparum* parasite counts

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				

			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 21				
Study number: Patient study number: Date of visit: dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: follow-up day 28				
Study number: Patient study number: Date of visit: dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: day __ (any other day that is not part of regular follow-up)				
Study number: Patient study number: Date of visit : dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/□l: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: follow-up day 35				
Study number: Patient study number: Date of visit : dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: day 42				
Study number: Patient study number: Date of visit: dd/mm/yyyy				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes which species ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor and other relevant institutions.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	

			☐ Yes ☐ No	

Case report form: final day of follow-up (28 or 42) (page 2)

Overall assessment

Outcome:

☐ adequate clinical and parasitological response
☐ early treatment failure
☐ late clinical failure
☐ late parasitological failure
☐ lost to follow-up
☐ withdrawn (complete section below: Reason for withdrawal)

Outcome occurred on follow-up day: (e.g. 1, 2, 3, 7, 14, …)

PCR:

☐ *P. falciparum* recrudescence
☐ *P. falciparum* reinfection
☐ other species
☐ mixed with *P. falciparum* recrudescence
☐ mixed with *P. falciparum* reinfection
☐ unknown

PCR corrected results:

☐ adequate clinical and parasitological response
☐ early treatment failure
☐ late clinical failure
☐ late parasitological failure
☐ lost to follow-up
☐ withdrawn

Reason for withdrawal:

Other comments:



APPENDIX 8. SERIOUS ADVERSE EVENT REPORT FORM

<table>
<tr><th colspan="3">Serious adverse event report form</th></tr>
<tr><td colspan="2">Health centre name:
Locality:
District:
Province:</td><td>Study number:
Patient study number:
Date of visit: dd/mm/yyyy
Follow-up day:</td></tr>
<tr><th colspan="3">Demographic data</th></tr>
<tr><td colspan="3">Date of birth: dd/mm/yyyy or estimated age: in: ☐ months or ☐ years
Height (cm): Weight (kg): Height (cm):

If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure
Provide the date of the last menstrual period: dd/mm/yyyy</td></tr>
<tr><th colspan="3">Serious adverse event</th></tr>
<tr><td colspan="3">Type of event: Severity Relationship to the study drug
☐ Death ☐ Mild ☐ None
☐ Life-threatening ☐ Moderate ☐ Possible
☐ Hospitalization or prolongation of hospitalization ☐ Severe ☐ Probable
☐ Permanent disability ☐ Life-threatening ☐ Definite
☐ Congenital anomaly or birth defect
Date of occurrence: dd/mm/yyyy</td></tr>
<tr><td colspan="3">Describe the serious adverse event (include all relevant laboratory results):</td></tr>
</table>

Describe how the reaction was treated:

Serious adverse event report form (page 2)
Comments (e.g. relevant medical history, drug allergies, previous exposure to similar drugs, other laboratory data, whether reaction abated after stopping the drug, whether reaction reappeared after reintroduction):

Outcome
☐ Recovered completely ☐ Not yet recovered ☐ Recovered with long-term consequences If patient recovered, provide date of recovery: dd/mmm/yyyy

Medicines (list the **medicine suspected of causing** the serious adverse event as well as all **concomitant medicines**)

Brand name, batch number, manufacturer name (list suspected medicine first)	Daily dose	Route	Start date	End date	Indications for use
			dd/mm/yyyy	dd/mm/yyyy	
			dd/mm/yyyy	dd/mm/yyyy	
			dd/mm/yyyy	dd/mm/yyyy	
			dd/mm/yyyy	dd/mm/yyyy	
			dd/mm/yyyy	dd/mm/yyyy	

Reporting officer
Name:

Qualification:

Address:

Phone:

Fax:

Email:

Signature: Date: dd/mm/yyyy

APPENDIX 9. GUIDELINES FOR ANALYSIS OF RESULTS

End-point for day X (X = 28 or 42)	PCR-uncorrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response on day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7	Failure	Failure
Other species infection	Censored day of infection	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before withdrawal or protocol violation	Excluded from analysis

End-point for day X (X = 28 or 42)	PCR-corrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	**Proportion (per-protocol analysis)**
Adequate clinical and parasitological response at day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7		
• falciparum recrudescence*	Failure	Failure
• falciparum reinfection*	Censored day of reinfection	Excluded from analysis
• other species mixed with falciparum recrudescence	Failure	Failure
• other species mixed with falciparum reinfection	Censored day of reinfection	Excluded from analysis
• other species infection	Censored day of infection	Excluded from analysis
• undetermined or missing PCR	Excluded from analysis	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before protocol violation or withdrawal	Excluded from analysis

* WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations*.
Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

APPENDIX 10. INFORMED CONSENT FORM FOR PARENTS OR GUARDIAN OF PROSPECTIVE CHILDREN OR MINORS

Evaluation of artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin piperaquine for the treatment of uncomplicated malaria in Uganda

This informed consent form is for parents or guardians of children aged 6 months to 10 years who attend the study, who have been invited to participate in a study to evaluate the efficacy and safety of dihydroartemisinin-piperaquine, artemether-lumefantrine, artesunate-amodiaquine and pyronaridine-artesunate for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Dr. Adoke Yeka and Prof. Moses Kamya
Name of organization:	Infectious Diseases Research Collaboration
Name of sponsor:	Presidents Malaria Initiative (PMI)/Ministry of Health
Name of proposal and version:	Version 1.11 dated 18.3 .2021

This informed consent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the full informed consent form.

Part I: Information sheet

My name is ……., and I work for the Ministry of Health/Infectious Diseases Research Collaboration . We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to verify that the medicine, artemether-lumefantrine (AL), pyronaridine-artesunate (PA), artesunate-amodiaquine (ASAQ) and dihydroartemisinin-piperaquine (DP) are still effective for curing malaria. Patients will be randomized to either two of the three drugs in each site.

We are inviting children aged 6 months to 10 years living in this area to take part in this study.

I am going to give you information and invite you to consent to have your child participate in this study. Before you decide whether you agree for your child to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Your decision to have your child participate in this study is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services your child receives at this clinic will continue and nothing will change. If you choose your child should not participate in this project, we will offer the treatment that is routinely provided in this clinic

for malaria: AL or ASAQ. You may change your mind later and stop participating even if you agreed earlier.

Your child will be treated with either artemether-lumefantrine (6 doses over 3 days given twice daily), pyronaridine-artesunate (3 doses over 3 days), artesunate-amodiaquine (3 doses over 3 days) or dihydroartemisinin-piperaquine (3 doses over 3 days), drugs currently accepted for treatment of malaria by the Uganda Ministry of Health. About 200 to 300 participants shall be involved in this study at each study site. The treatment that your child will receive will be determined by a process of randomization. Randomization means that the treatment given to your child will be based on chance. The chance of being placed into each of the treatment groups is the same. You will not be told which treatment your child has been assigned to receive. As the parasites that cause malaria can become resistant to the medicine, the Ministry regularly does studies to make sure these medicines are still working. You should know that like other antimalarial medicines, these medicines may cause some side-effects such as follows:

- Artemether-lumefantrine : Abdominal pain, asthenia, cough, diarrhoea, dizziness, fever, headache, joint and muscle pain, loss of appetite, rush, nausea, vomiting.
- Pyronaridine-artesunate : Abdominal pain, headache, asthenia, cough, diarrhoea, loss of appetite, nausea, vomiting.
- Dihydroartemisinin-piperaquine : Asthenia, cough, diarrhoea, fever, loss of appetite, nausea, vomiting.
- Artesunate-amodiaquine : Abdominal pain, asthenia, cough, diarrhoea, dizziness, insomnia, loss of appetite, nausea, vomiting.

These effects are usually minor and resolve quickly.

In the event we find that the medicine is not working, we will use what is called ‘rescue medicine’. This medicine is called quinine tablets, 10mg per kg body weight, given every 8 hours for 7 days according to current national recommendations. You should know that this medicine, like other antimalarial medicines, may cause some side-effects such as: tinnitus, impaired high tone hearing, headache, nausea, dizziness and dysphoria, and sometimes disturbed vision. These side effects are usually minor and resolve quickly.

The study will take place over 28 or 42 days. During that time, your child will have to come to the health facility for 1 hour each day for the first 3 days and then every week for 6 weeks according to the scheduled dates given to you. At the end of 6 weeks, the study will be finished. At each visit, your child will be examined by a physician. You may stay with your child during each of the visits and during the procedures.

Today, we will take blood for testing and your child will receive the first dose(s) of treatment.

On the

- 2nd visit: you will receive the 2nd dose(S) of treatment plus a blood test;

- 3rd visit: you will receive the 3rd dose(S) of treatment plus a blood test;
- 4th, 5th, 6th, 7th, 8th, 9th and 10th visits, you will have a blood test.

For the blood test, a small amount of blood, 4 (four) drops, will be taken from your child's fingertip or heel. An additional 1ml of blood will be collected from the arm on the seventh day to study the level of lumefantrine in the child's body. Your child may experience a bit of pain or fear when the finger or the heel is pricked. The pain should disappear within 1 day. The blood will be dropped onto a slide and a small piece of paper. The blood samples will be used to study the malaria in your child's blood. Several tests may be carried out on the blood samples including knowing how the parasites escape drug treatment or detection with the diagnostic tests and level of drugs use for treatment of malaria.

The examination of some of the blood samples will only be done after the study and it will not affect the success of the treatment. Nothing else will be done with the blood. The blood samples will be destroyed after the study when no more verification of the information collected is needed unless you consented for future biological use of your blood.

If you do not attend the scheduled visit, we will visit you at home by a member of the team.

As already mentioned, this medicine may have some minor side effects. It is also possible that it may cause some problems that we are not aware of; however, we will follow your child closely and keep track of these effects, if they arise, and of any other problems. We will give you a telephone number to call if you notice anything out of the ordinary or if you have concerns or questions. You can also bring your child to this health facility at any time and ask to see the study clinician. If your child experiences side-effects, we may use some other medicine, free of charge, which will help to reduce the symptoms or reactions, or we may stop one or more of the medicines. If this is necessary we will discuss it together. You and your child will always be consulted before we move to the next step.

Your child's participation will help us to make sure the medicine is still working, and this will benefit society and future generations. If you decide to participate in this study, the malaria treatment and/or any illnesses related to malaria will be given at no charge to you. We will give you on average 15,000 shillings to pay for your travel expenses to the clinic.

We will not share the identity of participants in the study with anyone. The information that we collect from this study will be kept confidential. Any information collected about your child will have a number on it instead of your child's name. Only the study team members will know what the number is, and we will lock that information up.

We will share the knowledge that we get from this study with you before it is made available to the public. Confidential information will not be shared. Afterwards, we will publish the results and make them available so that other interested people may learn from our study.

This proposal has been reviewed and approved by Makerere University School of Biomedical Sciences IRB, the Ugandan National Council of Science and Technology and the National drug Authority. This is a committee that makes sure that study participants are protected from harm.

Alternatives to participation

Your child's participation in this study is completely voluntary. If you decide you do not want to participate in the study, your child will be treated with the current standard of care in Uganda. The current standard of care of the treatment of uncomplicated malaria in Uganda is artemether-lumefantrine. If you decide to withdraw your child from the study at any time and for any reason, this will not affect your child's care at the outpatient department, where standard care for all medical problems is available. During the study, you will be informed promptly of any new information that may influence your willingness to continue participation in the study.

Withdrawal

Should you or your study doctors decide to withdraw your child from the study, your child will still be eligible for care according to the standard clinic procedures.

Use of the results

The findings from this study may be published in a medical journal. The study participants will not be identified by name. After the study is completed, you may request an explanation of the study results.

Treatment and compensation for injury

If your child is injured or you have questions about injuries as a result your child being in the study, please contact the doctors in the study clinic. The study staff shall take care of participants who get injuries as a result of their participation in the study. The services at the public health facility will be open to you in case of any other injury.

Questions

This study has been explained to you by the person who signed below and your questions were answered. If you have any other questions about the information here, you may call the Principal Investigator Dr Adoke Yeka of Infectious Diseases Research Collaboration (IDRC) on 077-2-473533. You may also contact Dr. Erisa Mwaka (0752-575050) the Chairman Makerere University School of Biomedical Sciences Research and Ethics Committee for questions related to participant`s rights and research related harm.

Part II: Certificate of consent

I have been invited to have my child participate in a study of a medicine used to treat malaria.
I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to my child's participation in this study.

Print name of child: _____
Print name of parent or guardian: _____
Signature of parent or guardian: _____
Date: _____
dd/mm/yyyy

Witness' signature: A witness' signature and the thumbprint of the participant's parent or guardian are required only if the parent or guardian is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant's parent or guardian and should have no connection with the study team.
I have witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of witness: _____
Signature of witness: _____
Date: _____
dd/mm/yyyy

and thumbprint of parent or guardian:



Investigator's signature:
I have accurately read or witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of investigator: _____
Signature of investigator: _____
Date: _____
dd/mm/yyyy

A copy of this informed consent form has been provided to participant's parent or guardian. _____ (initials of the principal investigator/assistant).
An informed assent form will _____ or will not _____ be completed.

APPENDIX 11. INFORMED ASSENT FORM FOR PROSPECTIVE CHILDREN OR MINORS

Evaluation of artemether-lumefantrine, pyronaridine – artesunate, artesunate-amodiaquine and dihydroartemisinin –piperaquine for the treatment of uncomplicated malaria in Uganda

This informed assent form is for children aged 8–10 years who, who have been invited to participate in a study to evaluate the efficacy and safety of artemether-lumefantrine, pyronaridine-artesunate and dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Dr. Adoke Yeka and Prof. Moses Kamya
Name of organization:	Infectious Diseases Research Collaboration
Name of sponsor:	Presidents Malaria Initiative (PMI)/Ministry of Health
Name of proposal and version:	Version 1.11 dated 18.3.2021

This informed assent form has two parts:

- Information sheet (to share information about the study with you)
- Certificate of assent (for signatures if you agree to take part)

You will be given a copy of the full informed assent form.

Part I. Information sheet

My name is ……, and I work for Ministry of Health/Infectious Diseases Research Collaboration. We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine, artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin-piperaquine are still effective for curing malaria.

We are inviting children aged 6 months to 10 years living in this area to take part in this study. I am going to give you information and invite you to participate in this study. You can choose whether you want to participate. We have discussed this study with your parent(s) or guardian, and they know that we are also asking you for your agreement. If you decide to participate in the study, your parent(s) or guardian also have to agree. If you do not wish to take part in the study, you do not have to, even if your parents have agreed. It is your choice. If you decide not to participate, nothing will change; this is still your clinic. Even if you say 'Yes' now, you can change your mind later and it will still be okay. You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. There may be some words you do not understand or things that you want me to explain more because you are interested or concerned. Please ask me to stop at any time, and I will take time to explain.

Interviewer: I have checked with the child, and he or she understands that participation is voluntary. _____ (initials)

You will be treated with either artemether-lumefantrine (6 doses over 3 days given twice daily), pyronaridine-artesunate (3 doses over 3 days), artesunate-amodiaquine (3 doses for 3 days) or

dihydroartemisinin-piperaquine (3 doses over 3 days), all current treatments recommended by the Ministry of Health of Uganda. The Ministry regularly conducts studies to make sure the medicine is still working. These medicines are known to be very effective, but you should know that they may have some minor side-effects such as follows:

- Artemether-lumefantrine: abdominal pain, asthenia, cough, diarrhoea, dizziness, fever, headache, joint and muscle pain, loss of appetite, rush, nausea, vomiting.
- Pyronaridine-artesunate: abdominal pain, asthenia, cough, diarrhoea, loss of appetite, nausea, vomiting.
- Dihydroartemisinin-piperaquine: Asthenia, cough, diarrhoea, fever, loss of appetite, nausea, vomiting.
- Artesunate-amodiaquine : Abdominal pain, asthenia, cough, diarrhoea, dizziness, insomnia, loss of appetite, nausea, vomiting.

These effects are usually minor and resolve quickly

The study will take place over 28 or 42 days. During that time, you will have to come to the health facility for 1 hour each day for the first 3 days and then every week for 4 or 6 weeks according to the scheduled dates given. At the end of 4 or 6 weeks, the study will be finished.

A small amount of blood will be taken from your finger, today and once at each visit during the follow-up. You may experience a bit of pain or fear when your finger is pricked. The blood will be dropped onto a slide and a small piece of paper. The blood samples will be used to study the malaria in your blood. The examination of some of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with your blood and it will be destroyed unless you consent to have it stored securely without personal information for future studies. An additional 1ml of blood will be collected from your arm on the seventh day to study the level of drugs in your blood.

Interviewer: I have checked with the child, and he or she understands the procedures. _____ (initials)

The medicine can have some unwanted effects or some effects that we are not currently aware of; however, we will follow you closely and keep track of any unwanted effects, if they arise, or any other problems. If anything unusual happens to you, we need to know, and you should feel free to call us any time with your concerns or questions. If you get sick or have concerns or questions between scheduled visits to clinic, you should let me or the other study staff know. You do not have to wait for a scheduled visit. We have also given your parents information about what to do if you are hurt or get sick during the study.

Interviewer: I have checked with the child, and he or she understands the risks and discomforts. _____ (initials)

If you decide to participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your participation will help us to make sure the medicine is still working, and this will benefit society and future generations.

Interviewer: I have checked with the child, and he or she understands the benefits. _____ (initials)

Because you live quite far from the clinic, we will give your parents or guardian enough money to pay for the trip here and back. We will not tell other people that you are participating in this study, and we will not share information about you with anyone who does not work in the study. Information about you that will be collected from the study will be put away, and no one but the study team will be able to see it. Any information about you will have a number on it instead of your name. Only the study team will know what your number is, and we will lock that information up.

When we have finished the research, I will sit down with you and your parent or guardian and tell you about what we learnt. Afterwards, we will be telling more people, scientists and others, about the study and what we found. We will do this by writing and sharing reports and data and by going to meetings with people who are interested in the work we do.

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby, you can come and see us. If you want to talk to someone else whom you know, like your teacher, doctor or auntie, that is okay too.

Part II: Certificate of assent

I have been invited to participate in a study of the efficacy of antimalarial medicines. I have read this information (or had the information read to me), and I understand it. I have had my questions answered and know that I can ask questions later if I have them. I agree to take part in the study. _____ (initials)

or I do not wish to take part in the study and I have not signed the assent below. _____ (initials)

Child's signature (only if the child assents):

Print name of child: _____

Signature of child: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the child's thumbprint are required only if the child is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____

Signature of witness: _____

Date: _____
(dd/mmm/yyyy)

and thumbprint of the child or minor:



Investigator's signature:

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
(dd/mmm/yyyy)

A copy of this informed assent form has been provided to the participant. _____ (initials of the principal investigator or assistant).

APPENDIX 12. INFORMED CONSENT FOR STORAGE OF BIOLOGICAL RESEARCH MATERIALS

Evaluation of artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin -piperaquine

This informed consent form is for the storage of biological research materials in a study to evaluate the efficacy and safety of artemether-lumefantrine, pyronaridine-artesunate, artesunate-amodiaquine and dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Dr. Adoke Yeka and Prof. Moses Kamya
Name of organization:	Infectious Diseases Research Collaboration
Name of sponsor:	Presidents Malaria Initiative (PMI)
Name of proposal and version:	Version 1.11 dated 18.3.2021

This informed consent form has two parts:

- Information sheet (to share information about the study with you)
- Certificate of assent (for signatures if you agree to take part)

You will be given a copy of the full informed assent form.

1. Introduction

While your child is in this study, blood samples (filter paper samples, plasma, serum and blood film) may be taken that may be useful for future research. These samples will be stored long-term at Makerere University IDRC molecular research laboratory. If you allow blood samples to be collected for future research, personal information will be removed and the samples will be securely stored for future testing of nay malaria related question. Samples may also be shared with investigators at other institutions.

2. What samples will be used for

The blood and the malaria parasites in it will be used to study malaria and the response of this disease to treatment. Results of these studies will not affect your care.

- These samples will be used for future research to learn more about malaria and other diseases.
- Samples will be used for additional medical/scientific project that may relate to malaria disease and associated potential investigational medicine. This may include research to help develop ways to detect, monitor or treat malaria disease.
- Samples will be used only for research and will not be sold or used for the production of commercial products.
- Genetic research may be performed on samples. However, no genetic information obtained from this research will be placed in the medical records. These samples will be identified only by codes so that they cannot be readily identified with the participant.
- For any future studies done on the samples not related to the current study, permission shall first be sought from the Makerere University School of Biomedical Sciences Research

and Ethics Committee Institutional Review Board and the Uganda National Council of Science and Technology.

3. Quantities of samples to be stored
Three blood filter paper sample spots collected at each point of collection throughout the study will be stored.

4. For how long will the samples be stored?
Samples could be stored for up to 5 years (some samples may be stored for a much less time)

5. Where will the samples be stored (full physical address, telephone contact, and email address of the location)
Samples will be stored at Makerere University IDRC Molecular Research Laboratory in Kampala, Uganda. The laboratory points of contact are stated below:
Names : Samuel Nsobya
Phone : 0752900068; Fax +414 540524
Website: http://idrc-uganda.org/: Email: admin@idrc-uganda.org
Physical address: Makerere University IDRC Molecular Research Laboratory, Mulago hill road.

6. Level of identification
Your samples will be coded so that your child's name cannot be readily identified. Reports about research done with the samples will not contain your child's name and we will keep the medical records confidential to the best of our ability.

In the future, researchers studying your child's samples may need to know more about your child, such as information about age and gender. If this information is already available because of your child's participation in the study, it may be provided to the researcher. Your child's name or anything that might identify you child personally will not be provided. You will not be asked to provide additional consent.

7. Risks
There are few risks to your child from future use of the samples. A potential risk might be the release of information from your child's health or study records. Reports about research done with your child's samples will not be put in the health record, but will be kept with the study records. The study records will be kept in a lockable filling cabinet place in a lockable room and only study staff will be able to access the record.

8. Benefits
There will be no direct benefit to your child. From studying your child's samples we may learn more about malaria or other diseases: how to prevent them, how to treat them, and how to cure them.

9. Research results/medical records

1. Results from future research using your child's samples may be presented in publications and meetings but your child's names will not be identified.
2. Reports from future research done with your child's samples will not be given to you or the doctor. These reports will not be put in your medical record.

10. Questions
If you have any other questions about the information here, you may call the Principal investigator Dr Adoke Yeka of Infectious Disease Research Collaboration (IDRC) on 077-2-473533. You may also call Dr. Erisa Mwaka (0752-5755050), chairman of the Makerere University School of Biomedical Sciences Research and Ethic Committee for questions related to participant`s rights and research related harm.

11. Freedom to refuse
You can change your mind at any time about allowing your child's samples to be used for future research. If you do, contact the study doctors, then your child's samples will no longer be made available for research and will be destroyed. Whether or not you allow us to use your child's samples in future research will not have any effect on your child's participation in this study or future participation in other studies.

12. Disposal plan for the research materials after the expiry of the storage period.
Expired samples will be destroyed as per the disposing site policy.

PARTICIPANT'S DECLARATION:
I have been invited to have my child participate in a study of a medicine used to treat malaria. I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction.
(Tick only one of the options)

I, …….. □ accept/ □ do not accept that the research materials obtained from my child's body as a research participant be stored confidentially for future use.

Print name of child: _____

Print name of parent or guardian: _____

Signature of parent or guardian: _____

Date: _____
dd/mm/yyyy

Witness' signature: A witness' signature and the thumbprint of the participant's parent or guardian are required only if the parent or guardian is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant's parent or guardian and should have no connection with the study team.
I have witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of witness: _____ and thumbprint of parent or guardian:

Signature of witness: _____ | |

Date: _____
dd/mm/yyyy

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
dd/mm/yyyy

A copy of this informed consent form has been provided to participant's parent or guardian.
_____ (initials of the principal investigator/assistant).

APPENDIX 13: GUIDELINES FOR GRADING PATIENT SYMPTOMS, SIGNS AND LABORATORY FINDINGS.

Estimating Severity Grade

If the need arises to grade a clinical AE that is not identified in the AE grading table, use the category "Estimating Severity Grade".

Determining Severity Grade

If the severity of an AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

Definitions

Basic Self-care Functions: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement)

LLN: Lower limit of normal

NA: Not Applicable

Operative Intervention: Surgical OR other invasive mechanical procedures

ULN: Upper limit of normal

Usual Social & Functional Activities: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

SYMPTOMS				
PARAMETER	**Grade 1 MILD**	**Grade 2 MODERATE**	**Grade 3 SEVERE**	**Grade 4 LIFE THREATENING**
Temperature (axillary)	37.5-37.9□C	38.0-39.5□C	> 39.5□C	Sustained fever, equal or greater than 40.0□C for longer than 5 days
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	N/A
Fatigue/Malaise	Symptoms causing no or minimal	Symptoms causing	Symptoms causing	Incapacitating fatigue/malaise symptoms

	interference with usual social & functional activities	greater than minimal interference with usual social & functional activities	inability to perform usual social & functional activities	causing inability to perform basic self-care functions
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Unintentional weight loss	N/A	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding]
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Dysphagia/odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake
Vomiting	Transient or intermittent vomiting with no or minimal	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR	Life-threatening consequences (e.g., hypotensive shock)

	interference with oral intake		Aggressive rehydration indicated (e.g., IV fluids)	
Seizure	Seizure, generalized onset with or without secondary generalization, lasting< 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5 – 20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Cough	Transient-intermittent	Persistent-constant	Uncontrolled	Cyanosis, stridor, severe SOB
Pain (indicate body site)	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated
PHYSICAL FINDINGS				
PARAMETER	**Grade 1 MILD**	**Grade 2 MODERATE**	**Grade 3 SEVERE**	**Grade 4 LIFE THREATENING**
Jaundice	Slight yellowing of sclera and conjunctiva	Moderate yellowing of sclera, conjunctiva, mucous membranes	Severe yellowing of sclera, conjunctiva, skin	N/A
Pallor	Minimally pale conjunctiva, nail beds	Moderately pale conjunctiva, nail beds	Paper white conjunctiva, nail beds, palms	N/A
Respiratory (RESP)				
Dyspnea or respiratory distress	Wheezing OR minimal increase in	Nasal flaring OR Intercostal retractions OR	Dyspnea at rest causing inability to perform usual	Respiratory failure with ventilatory support indicated

	respiratory rate for age	Pulse oxmetry 90– 95%	social & functional activities OR Pulse oximetry < 90%	
CARDIOVASCULAR (CV)				
Hemorrhage (significant acute blood loss)	N/A	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≥ 10 cc/kg packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 10 cc/kg packed RBCs Indicated
Hypertension	N/A	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	N/A	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
GASTROINTESTINAL (GI)				
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Mucositis/stomatitis	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (e.g., aspiration, choking)

Pancreatitis	N/A	Symptomatic AND Hospitalization not indicated (other than emergency room visit)	Symptomatic AND Hospitalization indicated (other than emergency room visit)	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
SKIN – DERMATOLOGICAL				
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	N/A
Rash - Cutaneous reaction	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	N/A	N/A
Hypopigmentation	Slight or localized	Marked or generalized	N/A	N/A
MUSCULOSKELETAL (MS)				
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social &	Stiffness or joint swelling causing greater than minimal interference with usual social &	Stiffness or joint swelling causing inability to perform usual social &	Disabling joint stiffness or swelling causing inability to perform basic self-care functions

	functional activities	functional activities	functional activities	
NEUROLOGIC (NEURO)				
Altered Mental Status	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions
CNS ischemia	N/A	N/A	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with neurological deficit
Developmental delay	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting

Neuromuscular Weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
GENITOURINARY (GU)				
Urinary tract obstruction (e.g., stone)	N/A	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing lifethreatening consequences
ENDOCRINE/METABOLIC (ENDO)				
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	N/A

Diabetes mellitus	N/A	New onset without need to initiate medication OR Modification of current medications to regain glucose control	New onset with initiation of medication indicated OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar nonketotic coma)
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy (e.g., fat loss from the face, extremities, buttocks)	Detectable by study participant (or by caregiver for young children)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	N/A

OTHER CLINICAL SIGNS AND SYMPTOMS				
Symptom	**Grade 1 MILD**	**Grade 2 MODERATE**	**Grade 3 SEVERE**	**Grade 4 LIFE THREATENING**
Clinical adverse event	Symptoms causing no	Symptoms causing	Symptoms causing	Symptoms causing

NOT identified elsewhere in this grading table	or minimal interference with usual social & functional activities	greater than minimal interference with usual social & functional activities	inability to perform usual social & functional activities	inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild Bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)

LABORATORY PARAMETERS				
PARAMETER	**Grade 1 MILD**	**Grade 2 MODERATE**	**Grade 3 SEVERE**	**Grade 4 LIFE THREATENING**
HAEMATOLOGY				
WBC, decreased	2,000 – 2,500/mm3	1,500 – 1,999/mm3	1,000 – 1,499/mm3	< 1,000/mm3

Absolute neutrophil count (ANC)	1,000 – 1,300/mm3	750 – 999/mm3	500 – 749/mm3	< 500/mm3
Hemoglobin (Hb)	8.5 – 10.0 g/dL	7.5 – 8.4 g/dL	6.50 – 7.4 g/dL	< 6.5 g/dL
Platelets, decreased	100,000 – 124,999/mm3	50,000 – 99,999/mm3	25,000 – 49,999/mm3	< 25,000/mm3
BLOOD CHEMISTRY				
PARAMETER	**Grade 1 MILD**	**Grade 2 MODERATE**	**Grade 3 SEVERE**	**Grade 4 LIFE THREATENING**
ALT (SGPT)	56 – 113 U/L 1.25 – 2.5 x ULN	114 – 225 U/L 2.6 – 5.0 x ULN	226 – 450 U/L 5.1 – 10.0 x ULN	> 450 U/L > 10.0 x ULN
AST (SGOT)	56 – 113 U/L 1.25 – 2.5 x ULN	114 – 225 U/L 2.6 – 5.0 x ULN	226 – 450 U/L 5.1 – 10.0 x ULN	> 450 U/L > 10.0 x ULN
Bilirubin (Total)	1.3 – 1.8 mg/dL 1.1 – 1.5 x ULN	1.9 – 3.0 mg/dL 1.6 – 2.5 x ULN	3.1 – 6.0 mg/dL 2.6 – 5.0 x ULN	> 6.0 mg/dL > 5.0 x ULN
Creatinine	1.3 – 1.6 mg/dL 1.1 – 1.3 x ULN	1.7 – 2.2 mg/dL 1.4 – 1.8 x ULN	2.3 – 4.1 mg/dL 1.9 – 3.4 x ULN	≥ 4.2 mg/dL ≥ 3.5 x ULN
Glucose, serum, high Nonfasting	116 – 160 mg/dL	161 – 250 mg/dL	251 – 500 mg/dL	> 500 mg/dL
Potassium, serum, high	5.6 – 6.0 mEq/L	6.1 – 6.5 mEq/L	6.6 – 7.0 mEq/L	> 7.0 mEq/L
Potassium, serum, low	3.0 – 3.4 mEq/L	2.5 – 2.9 mEq/L	2.0 – 2.4 mEq/L	< 2.0 mEq/L
Sodium, serum, high	146 – 150 mEq/L	151 – 154 mEq/L	155 – 159 mEq/L	≥ 160 mEq/L
Sodium, serum, low	130 – 135 mEq/L	125 – 129 mEq/L	121 – 124 mEq/L	≤ 120 mEq/L
URINALYSIS				
Hematuria (microscopic)	6-10 RBC/HPF	>10 RBC/HPF	Gross, with or without clots OR RBC casts	Transfusion indicated
Proteinuria, random Collection	1 +	2-3 +	4 +	N/A

APPENDIX 14: STUDY BUDGET.

Budget summary	
TES Budget	**USD.**
Labor	126,942
Fringe benefits	24,410.7
Travel, Transportation and per diem	14,162
Equipment, vehicles and non-expendable equipment	3,600
Supplies, equipment and other expendables	19,600
Other direct costs	92,750
Total Costs	**281,465**
Indirect costs	28,146
Award fees	8,444
Total Budget	**318,055**

APPENDIX 15: TIME FRAME

Time frame for TES study												
Specific Activity	**Jan-21**	**Mar-21**	**June-21**	**Sept-21**	**Dec-21**	**Jan-22**	**Mar-22**	**June-22**	**Sept-22**	**Dec-22**	**Jan-23**	**Mar-23**
Protocol approvals (IRB, UNCST, NDA)												
Recruitment and training of field staffs												
Enrolment of study participants												
Data entry and data cleaning												