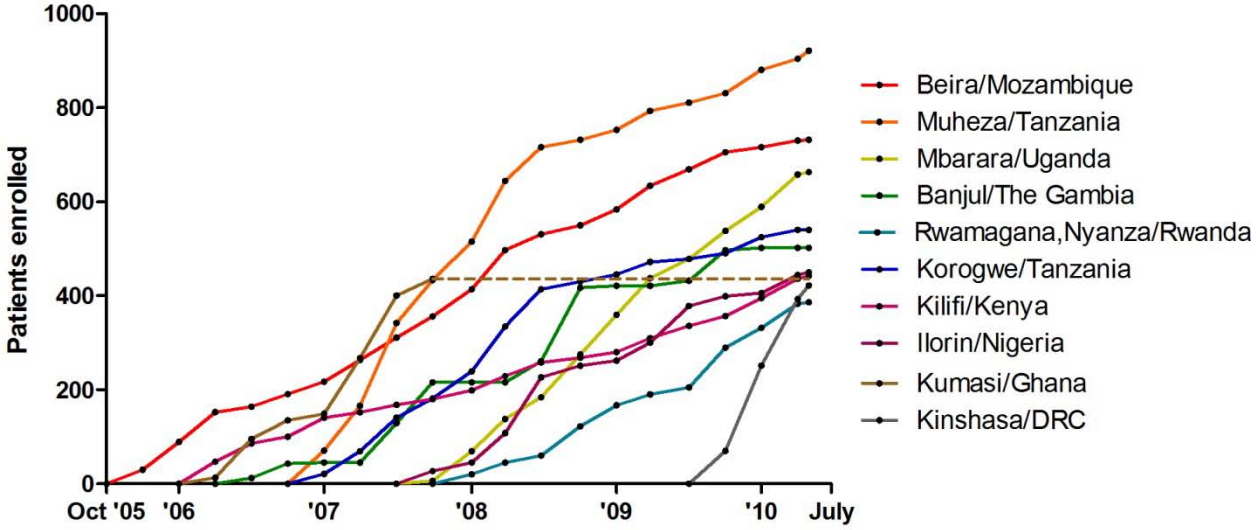
Supplemental material to "Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomized trial"

1. Enrollment history by site



2. Methods and findings Mortality Endpoint Review Committee

Method: The end-point review committee included one paediatrician with malaria experience and one clinical malariologist. The reviewers assessed the fatal cases independently from the trial investigators, and were blinded towards the study drug treatment allocations. All clinical and laboratory data (including those from the parasitology reference laboratory) were reviewed, along with the 'Severe Adverse Events' forms. Cases were only defined when both reviewers independently judged that a pathology other than malaria or its acute complications was the main cause of death.

Table 1. Cases with pathology other than malaria (or its acute complications) as a likely cause of death.

Age	Gender	Relevant details
6 y	F	Sudden onset of abdominal pain without fever. Death occurred within three hours of symptom onset. Parasitaemia 5/1000 RBC (no pretreatment)
22 m	M	Death followed return of consciousness and defervescence
3 y	F	Death followed return of consciousness and defervescence
7 m	M	History of diarrhoea, vomiting and generalized rash. Clinical diagnosis of measles. Parasitaemia 8/200 WC (one dose of pyrimethamine-sulfadoxine pretreatment)
3 y	M	Clinical diagnosis of tetanus. Parasitaemia 3/1000 RBC (no pretreatment)
11 m	F	Death followed return of consciousness and defervescence
21 m	M	Death followed return of consciousness and defervescence
5 y	F	Suspected meningitis. *Haemophilus influenzae* type b isolated from blood culture. Parasitaemia 2/1000 RBC (pyrimethamine-sulfadoxine pretreatment)
6 y	F	Clinical diagnosis of tetanus. Parasitaemia 3/1000 RBC (no pretreatment)
3 m	M	Clinical diagnosis of mastoiditis. Group A streptococcus isolated from blood culture. Parasitaemia 13/1000 RBC (no pretreatment)
4 y	M	Clinical diagnosis of left-sided pneumonia. *Haemophilus influenzae* type b isolated from blood culture. Parasitaemia 81/200 WC (two doses of quinine pretreatment)
22 m	M	Clinical diagnosis of myositis. Group A streptococcus isolated from blood culture. Parasitaemia 2/200 WC (three doses of quinine pretreatment)
14 m	F	Clinical diagnosis of severe acute malnutrition. *Salmonella* spp. isolated from blood culture. Parasitaemia 1/200 WC (no pretreatment)
6 m	M	Clinical diagnosis of severe cellulitis. Group A streptococcus isolated from blood culture. Parasitaemia 1/500 WC (six doses of artemether-lumefantrine pretreatment)
16 m	M	Clinical observation of pustular rash and impetigo (no coma). *Staphylococcus aureus* isolated from blood culture. Parasitaemia 3/200 WC (one dose of amodiaquine pretreatment)
14 m	M	Death followed return of consciousness and defervescence

3. Methods Neurological Endpoint Committee

Neurological sequelae were divided into 4 functional domains or systems, including motor deficits, vision deficits, hearing and speech deficits and persisting seizures. Severity of the deficits was graded according to the tables below. In case a patient had a pathological entry in 2 or more functional domains, the deficit with the most severe grade was moved up one 'severity grade' if the 'severe' grade had not yet been reached (Example 1: cerebellar ataxia (severe) + speech difficulties (mild) = total grade is severe. Example 2: Facial nerve palsy (moderate) + speech difficulties (mild) = total grade is severe). In case the clinical record form mentioned a pre-existing neurological problem and there was no significant deterioration of symptoms during the malaria episode, the neurological problems were not considered as being sequelae of the acute disease episode.

FUNCTIONAL SYSTEMS TABLES

1) MOTOR

CRF Entry	MILD	MODERATE	SEVERE
Monoparesis		x	
Hemiplegia/paresis			x
Quadriparesis			x
Continued posturing			x
Hypotonia	x		
Extrapyramidal rigidity			x
Cerebellar Ataxia			x
Gait * unsteady	x		
Gait* hemiplegic/ataxic			x
Gait* unable to walk			x
Cranial nerve palsies		x	
Facial nerve palsy		x	

* Gait was considered only in children > 18 months

IF there is more than one pathological entry within the motor system: the most severe grading prevails.

2) VISION

CRF entry	MILD	MODERATE	SEVERE
Blindness bilateral			x
Blindness unilateral		x	
Some impairment bilateral		x	
Some impairment unilateral	x		

3) HEARING and SPEECH

CRF entry	MILD	MODERATE	SEVERE
Deafness bilateral			X
Deafness unilateral		x	
Possible impairment bilateral		x	
Possible impairment unilateral	x		
Speech difficulties*	x		
Unable to speak*			X

*Speech was assessed only in children > 18 months

4) SEIZURES

In patients with no previous history of seizures:

Any seizures - moderate

4. Quality assessment of artesunate batches used in the trial

LC-MS/MS Analysis of the artesunate content in vials for injection

Methods[1]:

From each batch, 3 vials were selected for testing. Each vial's content was quantified using 3 replicate measurements. Samples were quantified using a standard curve constructed from 3 replicate samples at each calibration level (concentrations 19.2, 24.0, 28.8 ng/ml). Results for each vial and a batch average were summarized.

Sample preparation:

The content of each vial to be tested was reconstituted in 1.0-1.5 mL ethanol. The whole amount was transferred into a 250 ml volumetric flask thereby diluting the solution to 240 µg/ml (assuming initial content as stated; 60 mg). This solution was further diluted using volumetric flasks to a final approximate concentration of 24 ng/ml.

Quantification:

Samples were quantified using an API 5000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, USA). The final sample was injected into the LC-MS/MS system equipped with a TurboV ionization source (TIS) interface operated in the positive ion mode. Quantification was performed using selected reaction monitoring (SRM) for the transition m/z 402 – 163.

Results: The following results (table 1) were obtained for the different batches.

Conclusion: all tested vials' contents come within GMP specification of +/- 10% of stated content.

Reference.

Hanpithakpong W, Kamanikom B, Dondorp AM, Singhasivanon P, White NJ, Day NP, Lindegardh N. A liquid chromatographic-tandem mass spectrometric method for determination of artesunate and its metabolite dihydroartemisinin in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2008; 876: 61-8.

Site	Batch	Expire	pha ID	ARS (mg/vial)	Amount (%)	Average (%)	Batch average (%)
Mozambique	60109	Dec-08	A1 1	57.00	95.00	**95.28**	**95.28**
			A1 2	57.25	95.42		
			A1 3	57.25	95.42		
	ZA070701	Jun-10	B1 1	58.00	96.67	**96.94**	**93.79**
			B1 2	58.25	97.08		
			B1 3	58.25	97.08		
			B2 1	54.00	90.00	**90.00**	
			B2 2	54.25	90.42		
			B2 3	53.75	89.58		
			B3 1	56.75	94.58	**94.44**	
			B3 2	56.25	93.75		
			B3 3	57.00	95.00		
Kenya	ZA060903	Aug-09	C1 1	55.50	92.50	**92.50**	**93.84**
			C1 2	55.25	92.08		
			C1 3	55.75	92.92		
			C2 1	57.50	95.83	**95.28**	
			C2 2	56.75	94.58		
			C2 3	57.25	95.42		
			C3 1	56.00	93.33	**93.75**	
			C3 2	56.25	93.75		
			C3 3	56.50	94.17		
Tanzania Korogwe	ZA070406	Mar-10	D1 1	56.25	93.75	**92.36**	**91.53**
			D1 2	54.75	91.25		
			D1 3	55.25	92.08		
			D2 1	54.25	90.42	**90.55**	
			D2 2	54.25	90.42		
			D2 3	54.50	90.83		
			D3 1	55.50	92.50	**91.67**	
			D3 2	54.50	90.83		
			D3 3	55.00	91.67		
Tanzania Muheza	ZA070406	Mar-10	E1 1	62.25	103.75	**102.78**	**98.15**
			E1 2	61.25	102.08		
			E1 3	61.50	102.50		
			E2 1	55.25	92.08	**91.80**	
			E2 2	54.50	90.83		
			E2 3	55.50	92.50		
			E3 1	60.00	100.00	**99.86**	
			E3 2	60.00	100.00		
			E3 3	59.75	99.58		

Site	Batch	Expire	pha ID	ARS (mg/vial)	Amount (%)	Average (%)	Batch average (%)
The Gambia	ZA060203	Jan-09	F1 1	58.75	97.92	**97.78**	**96.76**
			F1 2	58.25	97.08		
			F1 3	59.00	98.33		
			F2 1	59.75	99.58	**100.00**	
			F2 2	59.75	99.58		
			F2 3	60.50	100.83		
			F3 1	55.50	92.50	**92.50**	
			F3 2	55.25	92.08		
			F3 3	55.75	92.92		
Uganda	ZA070406	Mar-10	G1 1	59.75	99.58	**100.14**	**92.50**
			G1 2	60.25	100.42		
			G1 3	60.25	100.42		
			G2 1	55.25	92.08	**92.08**	
			G2 2	55.50	92.50		
			G2 3	55.00	91.67		
			G3 1	51.25	85.42	**85.28**	
			G3 2	50.75	84.58		
			G3 3	51.50	85.83		
Nigeria	ZA070701	Jun-10	H1 1	54.75	91.25	**91.39**	**92.31**
			H1 2	54.50	90.83		
			H1 3	55.25	92.08		
			H2 1	57.50	95.83	**96.39**	
			H2 2	58.00	96.67		
			H2 3	58.00	96.67		
			H3 1	53.75	89.58	**89.17**	
			H3 2	53.25	88.75		
			H3 3	53.50	89.17		
Rwanda	ZA070701	Jun-10	I1 1	58.75	97.92	**97.64**	**95.00**
			I1 2	58.25	97.08		
			I1 3	58.75	97.92		
			I2 1	55.00	91.67	**92.50**	
			I2 2	55.50	92.50		
			I2 3	56.00	93.33		
			I3 1	56.75	94.58	**94.86**	
			I3 2	57.25	95.42		
			I3 3	56.75	94.58		
DRC	LA091001	10-08-12	J1 1	57.25	95.42	**95.00**	**95.88**
			J1 2	57.25	95.42		
			J1 3	56.50	94.17		
			J2 1	57.50	95.83	**96.25**	
			J2 2	58.00	96.67		
			J2 3	57.75	96.25		
			J3 1	57.50	95.83	**96.39**	

5. Quality assessment of quinine batches used in the trial

LC-UV Analysis of Quinine content in vials for injection

Methods (modified from ref. 1):
From each batch, 3 vials were selected for testing. Each vial's content was quantified using 3 replicate measurements. Samples were quantified using a standard curve constructed from 3 replicate samples at each calibration level (concentrations 15.7, 19.6 and 23.4 µg/ml). Results for each vial and a batch average were summarized.

Sample preparation:
The content of each vial to be tested was transferred into a 100 ml volumetric flask thereby diluting the solution to 6.00 mg/ml (assuming initial content 600 mg). Exactly 1000 µl of this solution was further diluted using a 250 ml volumetric flask to a final approximate concentration of 19.6 µg/ml.

Quantification:
Samples were quantified using a LaChrom Elite LC-UV system (Hitachi, Tokyo, Japan). Quantification was performed at the wavelength 250 nm.

Results: The following results (table 1) were obtained for the different batches.

Conclusion: all tested vials contained between 105-112% of stated content.

Reference.
1. Newton PN, Keeratithakul D, Teja-Isavadharm P, Pukrittayakamee S, Kyle D, White NJ. Pharmacokinetics of quinine and 3-hydroxyquinine in severe falciparum malaria with acute renal failure. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1999, 93:69–72.

Site	Batch	Expire	pha ID	QN (mg/vial)	Amount (%)	Average (%)	Batch average (%)
Mozambique	396	Mar-10	A1 1	627.56	104.59	105.42	106.06
			A1 2	634.11	105.68		
			A1 3	635.94	105.99		
			A2 1	633.62	105.60	106.58	
			A2 2	643.05	107.17		
			A2 3	641.76	106.96		
			A3 1	631.20	105.20	106.16	
			A3 2	633.65	105.61		
			A3 3	646.11	107.68		
	397	Sep-10	B1 1	641.64	106.94	108.58	108.71
			B1 2	667.79	111.30		
			B1 3	645.01	107.50		
			B2 1	635.42	105.90	107.38	
			B2 2	641.58	106.93		
			B2 3	655.88	109.31		
			B3 1	651.68	108.61	110.17	
			B3 2	672.84	112.14		
			B3 3	658.60	109.77		
Kenya	397	Sep-10	C1 1	639.47	106.58	108.21	108.50
			C1 2	650.55	108.42		
			C1 3	657.74	109.62		
			C2 1	646.35	107.73	108.98	
			C2 2	654.01	109.00		
			C2 3	661.30	110.22		
			C3 1	643.29	107.22	108.30	
			C3 2	658.85	109.81		
			C3 3	647.33	107.89		
Korogwe	396	Mar-10	D1 1	626.94	104.49	105.60	105.13
			D1 2	636.01	106.00		
			D1 3	637.81	106.30		
			D2 1	620.57	103.43	104.62	
			D2 2	624.74	104.12		
			D2 3	637.93	106.32		
			D3 1	625.14	104.19	105.18	
			D3 2	635.06	105.84		
			D3 3	632.97	105.50		
Muheza	396	Mar-10	E1 1	638.67	106.44	107.56	106.11
			E1 2	643.41	107.24		
			E1 3	647.27	107.88		
			E2 1	632.42	105.40	106.11	
			E2 2	638.76	106.46		
			E2 3	638.85	106.48		
			E3 1	627.80	104.63	105.04	
			E3 2	634.23	105.70		
			E3 3	628.72	104.79		
	398	Sep-10	F1 1	663.25	110.54	111.24	111.00
			F1 2	673.82	112.30		
			F1 3	665.31	110.88		
			F2 1	654.80	109.13	110.55	
			F2 2	672.38	112.06		
			F2 3	662.80	110.47		
			F3 1	660.68	110.11	111.20	
			F3 2	666.26	111.04		
			F3 3	674.71	112.45		
The Gambia	396	Mar-10	G1 1	661.39	110.23	112.04	107.14
			G1 2	676.70	112.78		
			G1 3	678.56	113.09		
			G2 1	620.76	103.46	104.40	
			G2 2	622.04	103.67		
			G2 3	636.37	106.06		
			G3 1	624.25	104.04	104.99	
			G3 2	627.28	104.55		
			G3 3	638.33	106.39		
Uganda	396	Mar-10	H1 1	628.72	104.79	106.29	105.96
			H1 2	631.81	105.30		
			H1 3	652.75	108.79		
			H2 1	628.50	104.75	105.30	
			H2 2	629.70	104.95		
			H2 3	637.26	106.21		
			H3 1	625.23	104.20	106.30	
			H3 2	649.94	108.32		
			H3 3	638.15	106.36		
	399	Sep-10	I1 1	658.82	109.80	110.60	111.91
			I1 2	663.38	110.56		
			I1 3	668.64	111.44		
			I2 1	658.66	109.78	111.64	
			I2 2	664.91	110.82		
			I2 3	685.88	114.31		
			I3 1	672.41	112.07	113.49	
			I3 2	676.39	112.73		
			I3 3	694.09	115.68		
Nigeria	396	Mar-10	J1 1	627.28	104.55	105.63	105.34
			J1 2	638.18	106.36		
			J1 3	635.85	105.98		
			J2 1	628.78	104.80	105.86	
			J2 2	628.84	104.81		
			J2 3	647.92	107.99		
			J3 1	620.05	103.34	104.52	
			J3 2	627.49	104.58		
			J3 3	633.77	105.63		
Rwanda	396	Mar-10	K1 1	620.12	103.35	104.12	104.03
			K1 2	622.17	103.69		
			K1 3	631.87	105.31		
			K2 1	614.24	102.37	103.55	
			K2 2	620.85	103.48		
			K2 3	628.78	104.80		
			K3 1	619.29	103.21	104.43	
			K3 2	627.59	104.60		
			K3 3	632.82	105.47		
	397	Sep-10	L1 1	639.59	106.60	107.30	107.26
			L1 2	636.53	106.09		
			L1 3	655.20	109.20		
			L2 1	635.09	105.85	107.00	
			L2 2	639.13	106.52		
			L2 3	651.80	108.63		
			L3 1	636.46	106.08	107.49	
			L3 2	645.34	107.56		
			L3 3	653.03	108.84		

6. Assessing pre-treatment: Classification of the efficacy of antimalarial drugs

Table 2.

Classification of antimalarials according to likely efficacy for the treatment of uncomplicated falciparum malaria in West Africa (Ghana, The Gambia, Nigeria) or the regions corresponding to the other AQUAMAT study sites. In the main paper, intermediate- efficacy and ineffective antimalarial drugs are grouped together as one category.

	Efficacy of pre-treatment: yes (y), no (n), intermediate (i)	
	Ghana, Gambia or Nigeria	Other study sites
quinine injection	y	y
artemether injection	y	y
artesunate/artemether tabs	y	y
sulphadoxin-pyrimethamine (SP)	i	n
SP-amodiaquine	y	i
chloroquine	n	n
amodiaquine	y	i
artemether-lumefantrine	y	y
artesunate suppository	y	y
artesunate-amodiaquine	y	y
artemether-amodiaquine	y	y
artemether-quinine	y	y
dihydroartemisinin (DHA)	y	y
DHA-amodiaquine	y	y
SP-artemether-lumefantrine	y	y
pyrimethamine-sulphamethopirazine	i	n

7. National and Sponsor Ethics Committee/Institutional Review Board (EC/IRB) approval documents for the AQUAMAT trial

Country	Study Site	Ethical Review Board	Address of ERB	Reference Number	Document	Date
DRC	**Kinshasa**	ESP UNIKIN Comité d'Ethique	Université de Kinshasa Faculté de Médecine BP 11850 Kinshasa DRC	ESP/CE/050/ 2009	Approval	24/09/2009
DRC	**Kinshasa**			ESP/CE/050B/ 2009	Revision	28/12/2009
Ghana	**Kumasi**	Committee for Human Research Publications and Ethics IRB00001567	University Office Kumasi Ghana	CHRPE/01/01 /06	Approval	23/01/2006
Ghana	**Kumasi**			CHRPE/01/01 /06	Renewal	22/05/2009
Kenya	**Kilifi**	KEMRI/ National Ethical Review Committee	KEMRI PO Box 54840 00200 Nairobi Kenya	SSC Protocol No 974 KEMRI/RES/ 7/3/1	Approval	21/10/2005
Kenya	**Kilifi**			KEMRI/RES/ 7/3/1	Renewal	29/07/2008
Kenya	**Kilifi**			KEMRI/RES/ 7/3/1	Renewal	06/07/2009
Kenya	**Kilifi**			KEMRI/RES/ 7/3/1	Renewal	18/08/2010
Kenya	**Kilifi**			KEMRI/RES/ 7/3/1	Revision	10/07/2008
Kenya	**Kilifi**			KEMRI/RES/ 7/3/1	Revision	21/05/2010
The Gambia	**Banjul**	The Gambia Government/ MRC Laboratories Joint Ethics Committee	c/o Laboratories Fajara PO Box 273 Banjul The Gambia	SCC1012	Approval	30/09/2005
Rwanda	**Rwamagana**	Rwanda National Ethics Committee (RNEC) IRB00001497/ FWA000001973	Ministry of Health PO Box 84 Kigali Rwanda	72/RNEC/ 2009	Approval	03/04/2008
Rwanda	**Rwamagana**			72/RNEC/ 2009	Renewal	18/06/2009
Rwanda	**Rwamagana**			127/RNEC/ 2009	Revision	02/11/2009
Rwanda	**Nyanza**			127/RNEC/	Approval	02/11/2009

Country	Study Site	Ethical Review Board	Address of ERB	Reference Number	Document	Date
				2009		
Nigeria	**Ilorin**	University of Ilorin Teaching Hospital Ethical Review Committee IRB00002974	PMB 1459 Ilorin Kwara State Nigeria	UITH/CAT/18 9/10/659	Approval	26/10/2007
Nigeria	**Ilorin**			UITH/CAT/18 9/10/659	Revision	14/02/2010
Mozambique	**Beira**	Comité Nacional de Bioética para a Saúde IRB 00002657	Ministério da Saúde C Postal 264 Av Eduardo Mondlane/Salvador Allende Maputo Moçambique	52/CNBS/05	Approval	23/06/2005
Mozambique	**Beira**			105/CNBS/07	Revision	04/06/2007
Tanzania	**Korogwe/ Muheza**	Tanzania Medical Research Coordinating Committee (MRCC)	National Institute for Medical Research P O Box 9653 Dar es Salaam Tanzania	NIMR/HQ/R 8a/Vol IX/435	Approval	29/05/2006
Tanzania	**Korogwe/ Muheza**			NIMR/HQ/R 8c/Vol IX/527	Renewal	26/02/2007
Tanzania	**Korogwe/ Muheza**			NIMR/HQ/R 8c/Vol I/22	Revision	20/04/2007
Tanzania	**Korogwe/ Muheza**			NIMR/HQ/R 8c/Vol I/60	Revision	15/08/2008
Uganda	**Mbarara**	Uganda National Council for Science and Technology FWA 00001293	Plot 6 Kimera Road, Ntinda PO Box 6884 Kampala Uganda	HS 349	Approval	26/09/2007
Uganda	**Mbarara**			HS 349	Renewal	05/09/2008
Uganda	**Mbarara**			HS 349	Renewal	31/08/2009
Uganda	**Mbarara**			HS 349	Revision	04/01/2010
UK	**Oxford**	University of Oxford OXTREC	University of Oxford Manor House The John Radcliffe Oxford OX3 9DZ United Kingdom	034-02	Approval	24/05/2005
UK	**Oxford**			034-02	Revision	03/10/2007
UK	**Oxford**			034-02	Revision	02/06/2008
UK	**Oxford**			034-02	Revision	11/08/2008
UK	**Oxford**			034-02	Revision	16/02/2009
UK	**Oxford**			034-02	Revision	09/03/2009

Country	Study Site	Ethical Review Board	Address of ERB	Reference Number	Document	Date
UK	**Oxford**			034-02	Revision	18/03/2009
UK	**Oxford**			034-02	Revision	08/06/2009
UK	**Oxford**			034-02	Revision	29/09/2009
UK	**Oxford**			034-02	Renewal	02/02/2010