



Study protocol for a randomised controlled double blinded trial of the dose-dependent efficacy and safety of primaquine for clearance of gametocytes in children with uncomplicated falciparum malaria in Uganda

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-002759
Article Type:	Protocol
Date Submitted by the Author:	19-Feb-2013
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Evidence based practice, Global health, Pharmacology and therapeutics, Research methods
Keywords:	INFECTIOUS DISEASES, Tropical medicine < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, PARASITOLOGY, PUBLIC HEALTH, Clinical trials < THERAPEUTICS

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TITLE

Study protocol for a randomised controlled double blinded trial of the dose-dependent efficacy and safety of primaquine for clearance of gametocytes in children with uncomplicated falciparum malaria in Uganda

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Keywords: Malaria, transmission, gametocyte, primaquine, glucose-6-phosphate dehydrogenase deficiency

1
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3 **Word count** - excluding title page, abstract, references, figures and tables: 3180 words
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10 **ABSTRACT**

11
12 Introduction For the purpose of blocking transmission of Plasmodium falciparum malaria from humans
13 to mosquitoes, a single dose of primaquine is recommended by the World Health Organisation as an
14 addition to artemisinin combination therapy. Primaquine clears gametocytes but causes dose-
15 dependent haemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency. Evidence is
16 needed to inform the optimal dosing of primaquine for malaria elimination programmes and for the
17 purpose of interrupting the spread of artemisinin-resistant malaria. This study investigates the efficacy
18 and safety of reducing doses of primaquine for clearance of gametocytes in participants with normal
19 G6PD status.
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31 Methods and analysis In this prospective, four-armed randomized placebo-controlled double-blinded
32 trial, children aged 1-10 years, weighing over 10kg , with haemoglobin \geq 8g/dL and uncomplicated
33 Plasmodium falciparum malaria are treated with artemether lumefantrine and randomised to receive a
34 dose of primaquine (0.1mg base/ kg, 0.4mg base/ kg or 0.75mg base/kg) or placebo on the third day of
35 treatment. Participants are followed up for 28 days. Gametocytaemia is measured by quantitative
36 nucleic acid sequence-based analysis on days 0, 2, 3, 7, 10 and 14 with a primary endpoint of the
37 number of days to gametocyte clearance in each treatment arm and secondarily the area under the
38 curve of gametocyte density over time. Analysis is for non-inferiority of efficacy compared to the
39 reference dose, 0.75 mg base/kg. Safety is assessed by pair-wise comparisons of the arithmetic mean
40 (+/- standard deviation) change in haemoglobin concentration per treatment arm and analysed for
41 superiority to placebo and incidence of adverse events.
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56 Ethics and dissemination Approval was obtained from the ethical committees of Makerere University
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3 School of Medicine, the Ugandan National Council of Science and Technology and the London School of
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5 Hygiene and Tropical Medicine. Results will be disseminated to inform malaria elimination policy,
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7 through peer-reviewed publication and academic presentations.
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11 **Trial registration:** NCT01365598
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13 14 **ARTICLE SUMMARY**

15 16 17 **Article focus**

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20 Single-dose primaquine, administered together with artemisinin combination therapy, blocks
21
22 transmission of *Plasmodium falciparum* malaria by clearing gametocytes.
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26 Primaquine, an 8-aminoquinoline, causes dose-dependent haemolysis in individuals with glucose-6-
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28 phosphate dehydrogenase (G6PD) deficiency. Evidence is lacking on the safety and efficacy of lower
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30 doses of primaquine.
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34 This is the protocol of a dose-finding trial being conducted in eastern Uganda.
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39 **Key messages**

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42 Dose-finding is a priority for the use of primaquine in malaria elimination programmes and to block the
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44 spread of artemisinin-resistant malaria.
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48 This trial is designed to investigate the efficacy and safety of reducing doses of primaquine for
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50 gametocytocidal action.
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3 This paper highlights the unique trial design issues that are relevant for investigating the efficacy and
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5 safety of antimalarials targeted against the sexual stages of malaria for blocking transmission rather
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7 than clinical cure.
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10 11 **Strengths and limitations of this study**

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14 For ethical reasons, in this trial, dose-finding is conducted in children with normal G6PD status, but,
15
16 ultimately, information is needed on the safety of lower doses in people with G6PD deficiency.
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20 This trial measures primaquine's transmission-blocking potential by assessing gametocyte clearance.
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22 Endpoints of mosquito transmission at multiple timepoints could be usefully assessed but on smaller
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24 numbers of individuals.
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27 **BACKGROUND**

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30 Sustained deployment of vector control measures and accessible, effective drug therapy has reduced
31
32 the transmission of *Plasmodium falciparum* in many endemic countries. However, further scaling-up of
33
34 currently available malaria control measures is unlikely to achieve malaria elimination in most
35
36 settings[1]. Moreover, the emergence of resistance to artemisinin in Southeast Asia[2 3], and the
37
38 development of insecticide resistance and adaptive behaviour in the mosquito vector[4-6] present
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40 significant threats to the current trend of declining malaria burden. Malaria elimination initiatives and
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42 artemisinin-resistance containment strategies both require additional tools that are specifically aimed at
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44 reducing the transmission of malaria parasites[7 8].
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53 Antimalarial drugs are designed primarily to target the asexual stages of the parasite that cause
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55 morbidity and mortality. The effect of antimalarial drugs on gametocytes, the transmission stages, has
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57 for decades been seen as ancillary. *P. falciparum* gametocytes undergo complex development that is
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3 characterised by five morphologically distinct stages of maturation[9]. The immature gametocyte stages
4
5 (I-IV) are sequestered in the reticuloendothelial system and bone marrow[10-12]. Mature stage V
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7 gametocytes typically appear approximately 12 days after the onset of patent asexual bloodstream
8
9 infection, and are the only gametocyte stage that circulates in the peripheral blood and is infective to
10
11 biting female *Anopheles* mosquitoes[13 14].The majority of antimalarial drugs, including artemisinins,
12
13 lumefantrine and piperaquine, have some efficacy against immature gametocytes[15 16]. These drugs
14
15 have the potential to reduce transmission at a population level because asexual parasites are cleared,
16
17 preventing *de novo* development of gametocytes, and fewer of the immature gametocytes that are
18
19 present upon initiation of treatment survive to maturity. However, the vast majority of symptomatic
20
21 cases have measurable and transmissible levels of mature gametocytes at presentation[17 18]. These
22
23 persist after treatment with all antimalarials that are currently implemented as first-line treatment,
24
25 including artemisinin combination therapy (ACT). Gametocytes that persist after ACT have repeatedly
26
27 been shown to be infectious to mosquitoes[17 19 20]. This post-treatment gametocyte carriage
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29 frequently occurs at low densities, commonly below the microscopic threshold for detection[21 22], but
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31 is sufficiently high for efficient mosquito infection[17 23].
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42 The only class of drugs that are effective against mature *P. falciparum* gametocytes is the 8-
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44 aminoquinolines. Primaquine is the most widely available drug in this class. The exact mechanism for
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46 this gametocytocidal activity is unknown, but it is probably dependent on oxidative damage to the
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48 intraerythrocytic parasite by primaquine metabolites[24] Primaquine as single dose of 0.75mg base/kg
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50 added to standard artemisinin containing therapy (ACT) has superior gametocytocidal activity to ACT
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52 alone[25-27]. All doses of primaquine described hereafter refer to the dose of primaquine base per unit
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54 weight. There are indications that doses of primaquine lower than 0.75mg /kg may be equally
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3 efficacious. A Thai study showed that both 0.5 and 0.25mg /kg of primaquine given with ACT to adults
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5 infected with malaria effectively and indistinguishably reduced the proportion of mosquitoes that
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7 became infected after a blood meal[28]. In small numbers of adults, total doses of 30mg and 15mg have
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9 shown comparable efficacy to a 45mg dose in reducing mosquito infection rates[29 30].
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17 The efficacy of primaquine when given as a single low dose is important in the light of concerns over the
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19 haematological safety of primaquine. There is conclusive evidence for primaquine-induced haemolysis
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21 in glucose-6 phosphate dehydrogenase (G6PD) deficient individuals[31 32]. G6PD deficient individuals
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23 are vulnerable to oxidative stress because their erythrocytes do not have alternative pathways for
24
25 G6PD-dependent NADPH production, which is essential to maintain anti-oxidant defences. There is
26
27 conflicting evidence on the risk of haemolysis after a single dose of primaquine. A single dose of 45mg
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29 primaquine given to a Vanuatan adult caused life-threatening haemolysis[33]. In G6PD deficient
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31 Tanzanian children, the mean fall in haemoglobin after a single dose of 0.75mg/kg primaquine was
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33 2.5g/dL (95% CI, 1.2 to 3.8 g/dl), though no associated severe adverse events were recorded and
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35 haemolysis was transient[34]. On the other hand, primaquine was reported to be well tolerated when
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37 0.75mg/kg was given without prior G6PD testing in large studies in Myanmar, Sudan, Russia, Cambodia
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39 and China[27 31 35 36].
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48 Because primaquine induced haemolysis is dose-dependent[29], and because gametocytocidal efficacy
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50 may be retained with primaquine doses lower than 0.75mg/kg, the World Health Organization (WHO)
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52 recommended dose in their 2010 Guidelines for the Treatment of Malaria, dose-finding studies are
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54 needed urgently. This trial tests the hypothesis that lower doses of primaquine have a substantially
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56 lower risk of, or an absence of, adverse effects but that their gametocytocidal efficacy is retained.
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METHODS AND ANALYSIS

Study design

The study is a prospective, randomised, parallel arm, placebo-controlled, double-blinded clinical trial of reducing doses of primaquine administered with artemether lumefantrine (AL) for the treatment of uncomplicated clinical *P. falciparum* malaria infection in children aged 1-10 years of age. The study uses a non-inferiority design to evaluate the efficacy and a superiority design to evaluate the safety of 0.1mg/kg and 0.4 mg/kg primaquine compared to 0.75mg/kg when added to AL.

Study objectives

1. To evaluate the efficacy of 0.1mg/kg, 0.4 mg/kg and 0.75 mg/kg primaquine when administered together with the 5th dose of AL as measured by gametocyte prevalence and density
2. To evaluate the safety of 0.1mg/kg, 0.4 mg/kg and 0.75 mg/kg primaquine when administered together with the 5th dose of AL as measured by change in mean haemoglobin, prevalence of severe anaemia (haemoglobin <5g/dL), and evidence of black urine (haemoglobinuria)
3. To assess the safety of different doses of 0.1mg/kg, 0.4 mg/kg and 0.75 mg/kg primaquine when administered together with the 5th dose of AL as measured by prevalence/ incidence of adverse events and tolerability

Participants and enrolment

The study is conducted at Walukuba Health Centre IV in Walukuba sub-county, Jinja district, in eastern Uganda. In this area, malaria transmission is year-round with two seasonal peaks. The entomological

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2
3 inoculation rate (EIR) was estimated at 7 infectious bites per person per year in Walukuba[37]. Study
4 participants are recruited from children attending the Health Centre IV with suspected malaria (Figure
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8 1). Inclusion criteria are: age 1-10 years, weight over 10kg, fever (tympanic temperature >38°C) or
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10 history of fever in the last 24 hours, *P. falciparum* mono-infection with a parasite density <500 000/ μ l,
11
12 and normal G6PD enzyme function. Exclusion criteria are: evidence of severe illness/ danger signs,
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14 known allergy to study medications, haemoglobin < 8g/dL , started menstruation, pregnancy or
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16 breastfeeding, antimalarials taken within the last 2 days, primaquine taken within the last 4 weeks,
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18 blood transfusion within the last 90 days.
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24 The fluorescent spot test[38] is used for G6PD screening. This test has a cut off of approximately 20%
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26 enzyme function; below that, there is no fluorescence. The WHO classification defines severe G6PD
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28 deficiency as 10% enzyme function[39].
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33 **Randomization, blinding and intervention**

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36 After enrolment (day 0), participants are randomised to a treatment arm stratified by gender (Figure 2).
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38 The study pharmacist selects sequential opaque envelopes (from either the male or the female pile).
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40 Each envelope contains a pre-determined treatment assignment code. The study pharmacist is the only
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42 member of the clinic team not blinded to the treatment arm and is not involved in assessing patients or
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44 assigning outcomes. All study site staff who administer drugs, assess patients and process laboratory
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46 samples do not have access to the randomization code breaker.
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52 All participants receive a three day course of artemether lumefantrine according to Ugandan national
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54 treatment guidelines for uncomplicated malaria. Participants are randomised to receive a placebo or a
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56 dose of 0.1mg/kg, 0.4 mg/kg or 0.75g mg/kg primaquine in addition to the AL treatment. The dose of
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3 primaquine/ placebo is given at the same time as the fifth dose of AL, in the morning of day 2. To
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5 preserve the accuracy of lower weight-based doses, all primaquine doses are administered in aqueous
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7 solution and measured using a sterile syringe. The placebo is aqueous solution alone. All doses including
8
9 placebo are mixed with a glucose syrup that masks the colour and taste of primaquine. All treatments
10
11 are directly observed. A snack with approximately 5g of fat is administered prior to both AL and
12
13 primaquine administration to optimise absorption of AL and minimise gastrointestinal side effects with
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15 primaquine. Participants are observed for 30 minutes; treatment is re-administered in any case of
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17 vomiting within this period. Repeated vomiting (>3 times) leads to exclusion from the study and
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19 treatment as complicated malaria according to national guidelines.
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27 Follow up measurements

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30 Study participants are reviewed on days 0, 1, 2, 3, 7, 10, 14, 21, 28 after enrolment or on any day of
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32 illness. On each of the scheduled visit days they are assessed clinically with standardised adverse event
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34 recording and blood samples are taken for microscopical detection of asexual parasites and
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36 gametocytes, molecular detection of gametocytes and haemoglobin measurements (Table 1).
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43 **Table 1 Summary of outcome measures**

	OUTCOME MEASURE	DESCRIPTION
EFFICACY		
	PRIMARY Mean number of days to gametocyte clearance (gametocyte clearance time, GCT)	Mean number of days per treatment arm for gametocytes to become undetectable using sub-microscopic molecular testing

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		methods (QT-NASBA). Re-appearance of gametocytes after day 14 will be considered re-infection and excluded.
SECONDARY	Mean (+/- SD) area under the curve of gametocyte density per day during 14 days of follow-up	Total number of gametocytes (measured by QT-NASBA) seen over follow up, averaged per day of follow up (days 0-14)
	Density of gametocytes on days 7, 10 and 14	Mean number of gametocytes (measured by QT-NASBA) per treatment arm on days 7, 10 and 14
	Proportion (%) of participants with gametocytes on each day of follow up	For each treatment arm, percentage of participants with gametocytes (measured by QT-NASBA) on each day of follow up from days 0-14.
SAFETY		
PRIMARY	Mean (+/- SD) maximal fall (+/ or -) in Hb (haemoglobin, g/dL) from enrolment to day 28 of follow-up	Mean maximal greatest negative difference in Hb (measured by HemoCue®) from enrolment value per treatment arm over 28 days follow up
SECONDARY	Follow-up day of Hb nadir	Mean day of follow up (day 0-28) per treatment arm of lowest Hb

		measurement (by Hemocue®)
	Maximal percentage fall in Hb level compared to enrolment value	Size of maximal Hb drop (by Hemocue®) during follow up (day 0-28) from enrolment value, divided by enrolment value, *100
	% participants with Hb < 5g/Dl during follow up	Percentage(number) per treatment arm during days 0-28
	Requirement for blood transfusion	Percentage (number) of children receiving blood transfusion per treatment arm during days 0-28
	Evidence of black urine	Percentage (number) of children with documented black/ dark urine with urine dipstick positive for Hb per treatment arm during days 0-28
	Incidence of serious adverse events by sign, symptom, laboratory parameter and relationship to taking study drug	Percentage (number) per treatment arm during days 0-28
	Incidence of gastrointestinal symptoms after taking study drug	Percentage (number) per treatment arm during days 2-7

Blood smears from all visits are Giemsa-stained and 100 microscopic fields are screened for asexual parasites on days 0, 1, 2, 3, 7, 10, 14, 21 and 28. Asexual parasites are counted against 200 white blood cells (WBC) or, if fewer than 10 parasites are observed per 200 WBC, against 500 WBC. Gametocytes are

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3 recorded if observed during this screening process. On day 0, 100 microscopic fields are re-read for
4 gametocytes specifically. If gametocytes are observed, they are quantified against 500 WBC. All
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8 microscopy readings are done by two independent microscopists, if they disagree on prevalence or if
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10 density results differ by more than 25%, a third reading is requested.
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16 Gametocytes are quantified on days 0, 2, 3, 7, 10 and 14 using quantitative real time nucleic acid
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18 sequence based analysis (QT NASBA), detecting and quantifying *Pfs25* mRNA. One hundred microlitre of
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20 finger prick blood is mixed with 900µL L6 guanidine buffer (Severn Biotech, UK) and stored at -80°C until
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22 automatic nucleic acid extraction by MagNAPure (Roche) using commercial high yield kits. The *Pfs25* QT-
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24 NASBA is specific for mature gametocytes with a sensitivity of 0.01-0.1 gametocytes/µL of blood when
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26 50µL blood samples are used for RNA extraction[40].
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34 Haemoglobin is measured on days 0, 1, 2, 3, 7, 10, 14, 21 and 28 using HemoCue 201+ photometers
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36 (HemoCue; Angelholm, Sweden). At each follow up visit, study staff assess participants in an objective
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38 manner according to a clinical record form and assessment for adverse events is conducted in a
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40 prospective, systematic fashion at all visits, including the enrolment visit (e.g. vomiting post AL). All data
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42 are double-entered in real time.
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48 **Safety considerations**

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51 A protocol was developed in order to standardise the detection, investigation and management of
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53 severe haemolysis in this trial (Figures 3 and 4). A Data Safety Monitoring Board (DSMB) has been
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3 installed; clinically relevant haemolytic events, hospital admissions, blood transfusions and deaths are
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5 reported within 72 hours to this DSMB.
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8 9 10 **Ethical considerations**

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13 The study protocol and informed consent forms were approved by the Makerere University School of
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15 Medicine Research Ethics Committee (protocol 2011-210), the Uganda National Council of Science and
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17 Technology (protocol HS1056) and the London School of Hygiene and Tropical Medicine research ethics
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19 committee (protocol 5987). The Ugandan National Drug Authority approved the protocol and
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21 importation of primaquine for the purposes of the study. The Data Safety Monitoring Board and Trial
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23 Advisory Committee for the study agreed to meet at predetermined stages of the study. Before the
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25 study began, local community stakeholders (including village health team and local council members) in
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27 Walukuba were consulted and a community advisory board meeting was held.
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36 **Sample size**

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39 For efficacy, the sample size calculation is based on non-inferiority of each of the two test dose arms to
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41 the comparator arm, the WHO-recommended dose of primaquine, 0.75mg/kg. The primary outcome
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43 measure is number of days to gametocyte clearance. The addition of primaquine (0.75mg/kg) to ACT in
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45 Tanzania reduced the time to gametocyte clearance from 28.6 to 6.3 days (standard deviation 6
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47 days)[41]. Allowing for a 10% loss to follow up, a sample size of 120 per arm will provide over 80%
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49 power at the 0.05 significance level to detect non-inferiority to the standard arm with a non-inferiority
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51 margin of 2.5 days, which was considered to be a clinically relevant reduction in gametocyte clearance
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53 time. This sample size also allows for an analysis of superiority of the efficacy of the two test dose arms
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55 to placebo.
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6 For safety, the sample size calculation is based on superiority of each of the two test dose arms to the
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8 comparator arm (0.75mg/kg). For this comparator arm, Shekalaghe *et al.*[34] found an overall mean
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10 absolute drop in haemoglobin by day 7 after treatment of 0.6g/dL (standard deviation 1.5). Therefore,
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12 with 80% power and at the 0.05 significance level, a sample size of 99 would be required to detect a
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14 difference in mean maximal drop in haemoglobin between treatment groups of 0.6g/dL.
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17 18 19 **Data analysis**

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22 Data will be double entered in Microsoft Access and imported into Stata version 12.0 (Statacorp Ltd,
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24 Texas, US). All efficacy analyses will be based on gametocyte detection by *Pfs25* QT-NASBA. Gametocyte
25
26 density on days 7, 10 and 14 will be compared with the comparator arm (0.75mg primaquine/kg) by chi-
27
28 square test. The mean duration of gametocyte carriage and 95% confidence interval will be estimated in
29
30 each treatment arm and compared with the comparator arm using a previously validated mathematical
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32 model[42]. The area under the curve (AUC) of gametocyte density over time will be calculated using the
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34 method described by Mendez *et al.*[43]. For individuals who are gametocyte positive at enrolment,
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36 Kaplan-Meier survival analysis will be used to compare the decline in gametocyte prevalence.
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39 The primary safety outcome, mean maximal fall in haemoglobin concentration during 28 days of follow
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41 up will be assessed for each treatment arm. Pair-wise comparisons will be made between each of the
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43 treatment arms and compared with the comparator arm using unpaired t-tests.
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51 **DISCUSSION**

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55 In the 2010 edition of the Guidelines for the Treatment of Malaria, the World Health Organisation
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57 (WHO) recommends that a single dose of 0.75mg/kg primaquine is added to ACT in malaria elimination
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3 programmes and for epidemic control, provided the risks of haemolysis in G6PD deficient patients are
4 considered. This guidance was recently updated to recommend a lower dose of 0.25mg/kg primaquine
5 without G6PD testing for new malaria elimination programmes and to prevent the spread of artemisinin
6 resistance[31]. The revision was based largely on grey literature and historical data rather than on
7 recent clinical trials and few of the data are in the public domain[44]. There have been no formal dose-
8 finding studies using contemporary tools and standards for the measurement of drug efficacy and safety
9 for the combination of ACTs and primaquine. In the current study, we aim to provide these urgently
10 needed efficacy data and provide safety data for individuals with normal G6PD enzyme function.
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26 Relatively few trials have been designed specifically to test gametocytocidal drugs *in vivo*. Standardised
27 protocols and trial designs for assessing the efficacy of drugs targeted against asexual parasites[45
28 46]are not suitable to assess gametocytocidal drugs, where the main outcome is transmission-blocking
29 activity rather than clinical or parasitological cure. There is no agreement on the best tools to quantify
30 gametocyte carriage. Many trials have used microscopy to measure gametocytes[26 27 47-49] while it
31 has been known for decades that microscopy is notoriously insensitive for detecting gametocytes[50].
32 Gametocytes typically circulate at densities that are $\leq 1\%$ of asexual parasite densities[16 51].
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42 Nevertheless, gametocytes are often simply recorded while screening for asexual parasites. If slides are
43 specifically read for gametocytes, the number of microscopic fields that is screened is mostly the same
44 as that for asexual parasites[52]. As a consequence, gametocytes measured by routine microscopically
45 underestimate the total gametocyte prevalence by up to 10-fold[16 17 21 22]. In the current study,
46 gametocytes are quantified with the most widely-used quantitative molecular gametocyte detection
47 method, QT-NASBA that has an estimated sensitivity of 0.01-0.1 gametocytes/ μL blood in the blood
48 sample taken[40]. The use of this sensitive molecular method will increase the power of our efficacy
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3 estimates since up to 90% of symptomatic malaria patients may harbour (submicroscopic) gametocyte
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5 densities prior to initiation of treatment[16].
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11 Gametocyte density is associated with the likelihood of mosquito infection and some of the lowest
12
13 gametocyte densities may therefore be unlikely to result in mosquito infections. In general, there are
14
15 limitations to which gametocyte prevalence or density can be used to predict mosquito infection rates.
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17 The fitness or infectivity of gametocytes is variable, especially after treatment[19 53 54]. Very early
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19 studies demonstrated that primaquine may render gametocytes non-infectious several days before they
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21 are cleared from the circulation[30 55 56]. The only approach to directly measure transmission blocking
22
23 potential involves assessing the infectiousness of the participant's blood to mosquitoes using the
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25 membrane feeding assay or direct skin feeding assays[57], the latter being described by early
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27 malariologists[58 59]. However, the capacity for mosquito feeding assays is not widely available and
28
29 repeated assessments of infectiousness on the same patients have never been performed as part of
30
31 clinical trials. This is partly because of ethical concerns related to repeated venous bleeding in young
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33 children, and partly because of the complexity of mosquito husbandry when large numbers of
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35 mosquitoes are required for robust transmission estimates[60]. In the absence of biomarkers, using the
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37 prevalence and density of gametocytes after treatment is the most pragmatic approach to assess the
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39 transmission blocking efficacy of drugs across a variety of malaria endemic settings.
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51 To assess the safety of the 8-aminoquinoline drugs, there must be a clear definition of the risk of
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53 haemolysis and how it should be measured[31 61]. The safety profile may best be defined by the
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55 incidence of endpoints that could compromise health, such as signs of severe haemolysis, and the need
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3 for interventions such as haematinic drug administration, hospitalization or blood transfusion. These
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5 events, however, are rare and changes in haemoglobin concentration may be a more sensitive primary
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7 safety outcome for standard clinical trials. In a recent Cochrane review of randomised controlled trials of
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9 primaquine's efficacy, only one trial[25] was found to have measured haemoglobin concentration to
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11 assess safety[62]. In this current study, clinically-relevant safety endpoints have been selected and a
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13 standardized procedure is in place for the investigation and management of severe haemolysis. A
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15 shortcoming of the current study is that safety data are most urgently needed in the most vulnerable
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17 group, G6PD deficient individuals. For ethical reasons this group was excluded. The authors consider
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19 that the priority is first to determine the minimal effective dose in a G6PD normal population before
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21 G6PD deficient individuals are exposed to this low dose of primaquine to assess safety.
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31 The ultimate evidence for a beneficial role of primaquine in reducing malaria transmission would come
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33 from trials assessing the effect of the drug on measures of community-level transmission. Once a safe
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35 and efficacious dose of primaquine in combination with ACTs is established, a next step is designing
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37 these community trials. Treatment of symptomatic cases could play an important role in reducing the
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39 spread of (resistant) malaria strains from symptomatic patients[63]. However, because of the large pool
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41 of asymptomatic parasite carriers in all endemic settings[64] and their importance in defining
42
43 transmission potential, any effect of primaquine on community-wide transmission will be limited if
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45 administration is restricted to symptomatic cases. Other strategies such as pro-active screening and
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47 treatment and (focal) mass drug administration may have a larger impact in some settings[65]. This trial
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49 forms the starting point for defining the optimal dose of primaquine for use in transmission-blocking
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51 interventions.
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Trial Status

Recruitment began on 6th December 2011. The trial is ongoing.

List of abbreviations

ACT artemisinin combination therapy

AL artemether lumefantrine

G6PD glucose-6-phosphate dehydrogenase

Hb haemoglobin

QT-NASBA quantitative real time nucleic acid sequence-based

WHO World Health Organization

Authors' contributions

Conceived and designed the study: ACE, SGS, SY, NJW, TB, CD. Participated in logistical planning: ACE, SGS, SY, EW, MK, NJW, TB, CD. Provided statistical support for the sample size estimates and the design of the statistical analysis: EW. Provided expertise for sub-microscopic gametocyte measurement: TB. Organised ethical applications, community sensitisation and study implementation: ACE. Wrote the manuscript: ACE, TB, CD. All authors read and approved the final manuscript.

Acknowledgements

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3 The authors would like to thank staff at the Infectious Diseases Research Collaboration (IDRC) in Uganda,
4 notably, Mr Moses Kiggundu, Dr Catherine Maiteki-Sebuguzi, the members of the Programme for
5 Resistance, Immunology, Surveillance and Modelling of Malaria in Uganda (PRISM) and the ACT PRIME
6 and PROCESS research teams and also Ms Carolynne Stanley for logistical support at the London School
7 of Hygiene and Tropical Medicine. Alice C. Eziefula is funded by a Clinical Fellowship from the Wellcome
8 Trust of Great Britain (#090558/Z/09/Z).
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17 **Competing interests**

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21 None declared
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FIGURE LEGENDS

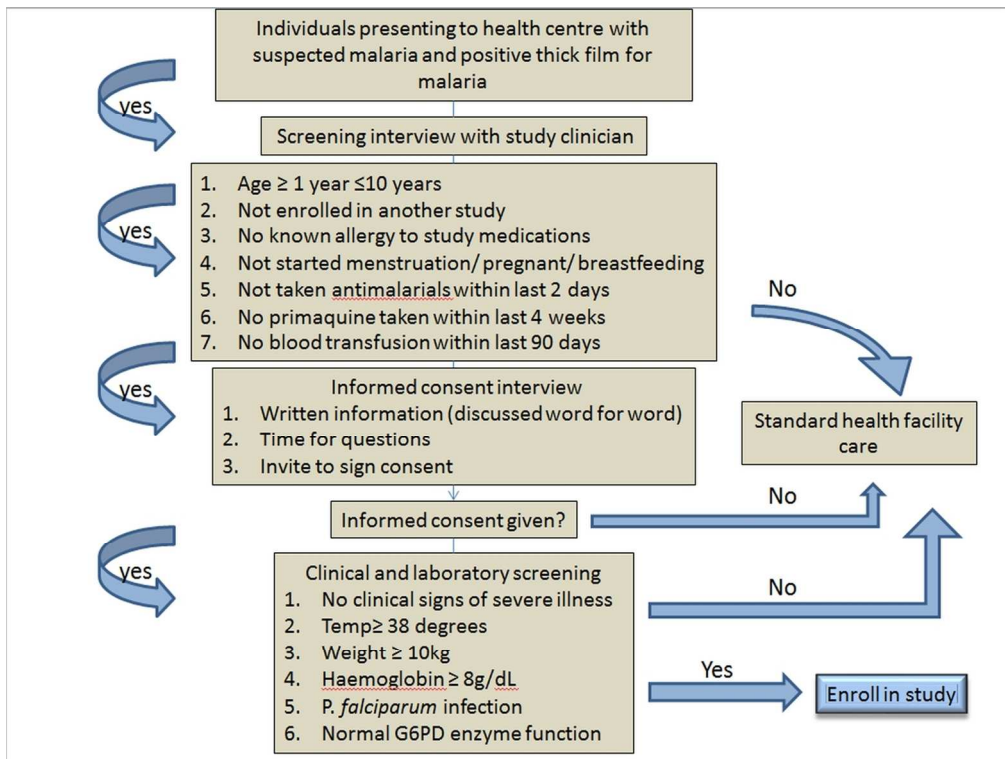
Figure 1: Enrolment and selection procedures

Figure 2: Participant flow diagram

Figure 3: Procedure for investigation of suspected haemolysis

Figure 4: Procedure for management of haemolysis

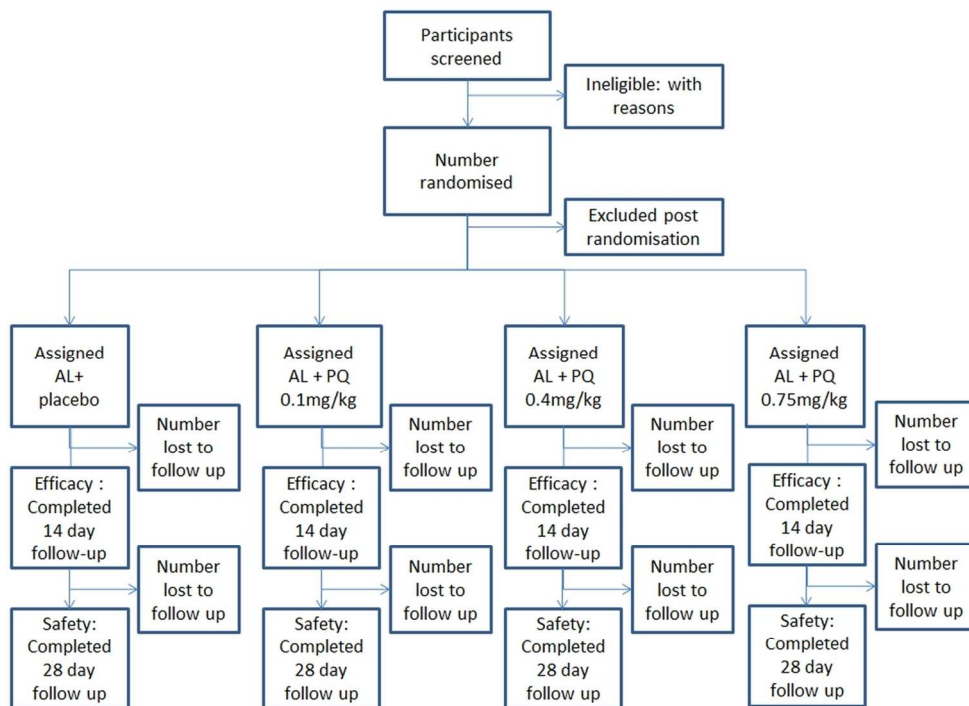
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Enrolment and selection procedures
119x90mm (300 x 300 DPI)

Review only

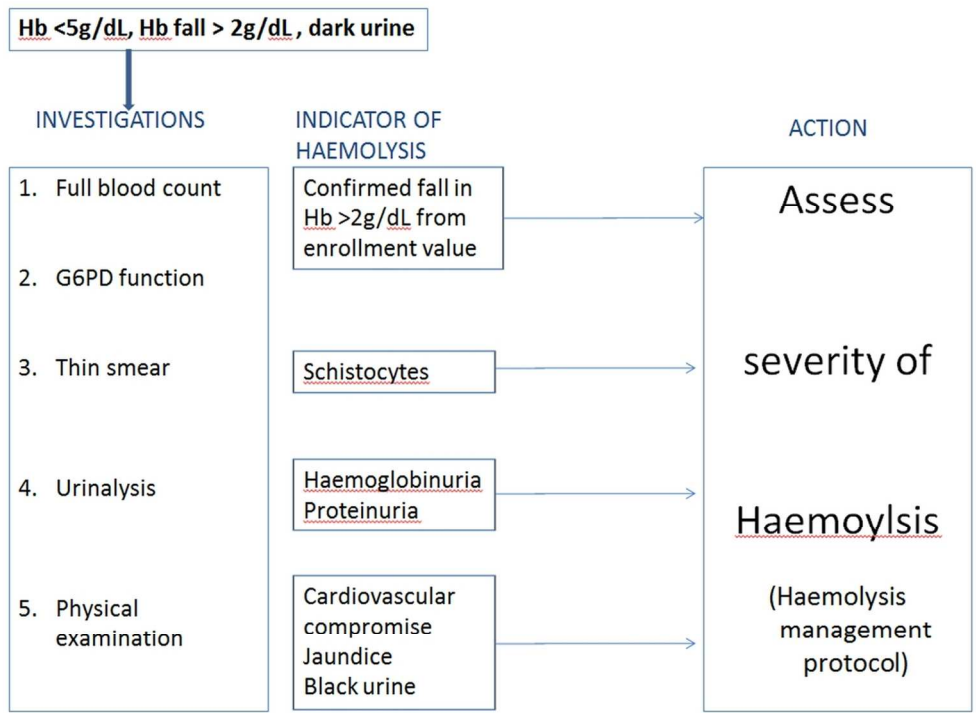
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Participant flow diagram
119x90mm (300 x 300 DPI)

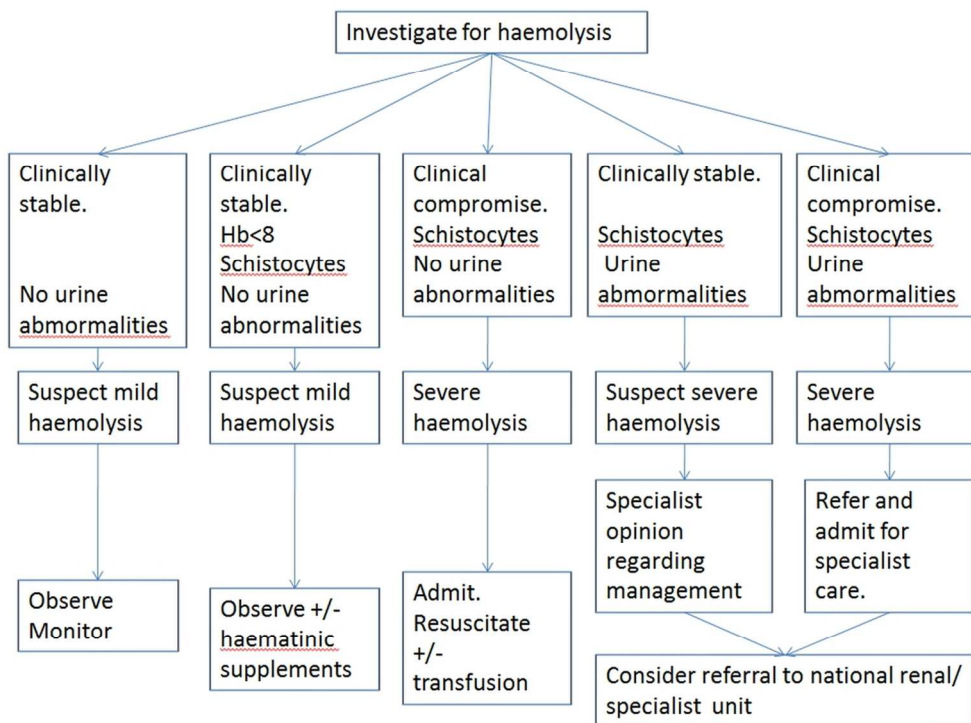
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Procedure for investigation of suspected haemolysis
120x90mm (300 x 300 DPI)

Review only



Procedure for management of haemolysis
119x90mm (300 x 300 DPI)

Review only