Review Article

Safety Considerations for Malaria Volunteer Infection Studies: A Mini-Review

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Abstract. Malaria clinical studies entailing the experimental infection of healthy volunteers with *Plasmodium* parasites by bites from infected mosquitos, injection of cryopreserved sporozoites, or injection of blood-stage parasites provide valuable information for vaccine and drug development. Success of these studies depends on maintaining safety. In this mini-review, we discuss the safety risks and associated mitigation strategies of these three types of experimental malaria infection. We aimed to inform researchers and regulators who are currently involved in or are planning to establish experimental malaria infection studies in endemic or non-endemic settings.

INTRODUCTION

Experimental infection of healthy volunteers with *Plasmodium* parasites mediated by bites from infected mosquitos, injection of cryopreserved sporozoites, or injection of blood-stage parasites is being increasingly carried out to assist development of malaria vaccines^{1–4} and drugs.^{5–7} As well, these studies provide valuable insights into parasite biology^{8,9} and the host–parasite interaction.^{8,10,11} These studies have been commonly referred to as controlled human malaria infection studies. The use of this term has been subject to recent debate. In this mini-review, we use the term malaria volunteer infection studies (VIS) to conform to the nomenclature adopted by the Medicines for Malaria Venture. As in other areas of research, the safe use and containment of infectious material and the mitigation of other potentially hazardous biological risks are essential to protect study subjects, staff conducting these studies, and the wider community. In this mini-review, we discuss the safety risks to these three groups and the techniques used to mitigate these risks and maintain the acceptability of malaria VIS conducted in endemic and non-endemic settings.

Types of malaria VIS. Of the three types of malaria VIS, infection induced by bites from an infected mosquito (mosquito challenge) represent the most natural method. *Anopheles* mosquitos, typically laboratory-reared *Anopheles stephensi*, are rendered infectious by feeding on in vitro–cultured *Plasmodium falciparum*, or in the case of *Plasmodium vivax* by feeding on infected patients because in vitro culture is not possible.¹² A less natural mode of infection is needle-based intravenous inoculation with aseptic purified cryopreserved sporozoites. Currently, only *P. falciparum* cryopreserved sporozoites are available, but it is possible cryopreserved sporozoites will become available for other *Plasmodium* species. Finally, induced blood-stage malaria (IBSM) involves the intravenous injection of malaria-infected red blood cells (RBC). Each method has safety risks and mitigation strategies (summarized in Table 1).

RISKS TO SUBJECTS

Cross infection. Human malaria parasite (HMP) banks are collections of malaria parasites contained within human blood products¹³; these can be used as the source of parasites for all three types of malaria VIS. Currently, in vitro culture is only possible for *P. falciparum*¹⁴ and *Plasmodium knowlesi*,^{15,16} with the latter not having been used for VIS in the modern era. In vitro culture permits the production of *P. falciparum* HMP banks under good manufacturing practice conditions, thereby permitting the selection of parasite strains for all types of *P. falciparum* VIS, and blood type for IBSM.^{13,17} HMP banks produced through continuous in vitro culture must be tested for adventitious agents as required by regulatory agencies.

HMP banks can be produced from donors (ex vivo from parasitemic volunteers infected via mosquito bites or from IBSM from existing banks, or by collection of parasitemic blood from naturally infected individuals [e.g., returned travelers with malaria]), providing donors are adequately screened. Donors should complete a lifestyle questionnaire to identify risk factors for transfusion-transmitted diseases (e.g., blood-borne viruses, prion diseases, Q-fever, leptospirosis, brucellosis, and Chagas disease). At our center, this questionnaire is based on the eligibility criteria for blood donation in Australia. Donors for HMP banks are screened, using sensitive polymerase chain reaction (PCR)-based and serology assays, for a wide range of blood-borne viruses (HIV 1 and 2, human T-lymphotropic virus, Epstein–Barr virus [EBV], cytomegalovirus [CMV], hepatitis C, hepatitis B, parvovirus B19, West Nile virus, Ross River virus, Barmah Forest virus, dengue fever, and human herpes virus 6 and 7). The presence of active blood-borne disease or serological evidence of latent infection that can reactivate (e.g., syphilis) is exclusionary. Leukodepletion of collected blood is routinely practiced and provides an additional level of security by removing cell-associated herpes viruses, such as CMV and EBV,¹⁸ that are carried in leukocytes. The absence of CMV and EBV is confirmed by PCR testing of the donor unit of blood, thus decreasing the need to match the serostatus of the donor and recipient for CMV and EBV. Because *P. vivax* cannot be cultured in vitro, mosquito bite inoculation can only occur if the mosquitos have been fed on infected donors. Therefore, these infected donors may require screening for other diseases transmitted by *An. stephensi* mosquitos, including lymphatic filariasis. Although filarial parasites could theoretically be transmitted by blood transfusion, microfilariae transmitted this

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TABLE 1
Summary of safety risks and mitigation strategies in malaria VIS

Safety risk	Type of VIS in which risk occurs	Mitigation strategies for risk
Transfusion-related infections	IBSM	Screening and microbial contamination testing of HMP bank and the challenge agent Leukodepletion of HMP bank
Coinfection with other malaria species	Mosquito, sporozoite, IBSM	Donors screened to exclude mixed malaria infection
Coinfection with filariasis	Mosquito (<i>P. vivax</i>)	Donors screened for filariasis
Transfusion reaction	IBSM	RBC antibody negative at screening Inoculation of subjects with a compatible blood group and rhesus type Small numbers of RBCs in the challenge agent
Alloimmunization	IBSM	Inoculation of subjects with a compatible blood group and rhesus type RBC antibody negative at the end of the study.
Higher malaria inoculation dose than planned	Mosquito, sporozoite, IBSM	Five infectious bites (mosquito) Controlled manufacturing process for consistent inoculation size (sporozoite ⁶⁷ and IBSM)
Cardiac inflammation	Mosquito, sporozoite, IBSM	Exclusion of subjects at increased risk of cardiovascular disease
Relapse from <i>P. vivax</i> hypnozoites	Mosquito (<i>P. vivax</i>)	Exclusion of subjects with low CYP2D6 and G6PD activity at screening Primaquine or tafenoquine treatment at the end of the study
Hemolysis from primaquine or tafenoquine treatment	Mosquito (<i>P. vivax</i>), sporozoite (<i>P. vivax</i>)	G6PD testing at screening
Exposure of study staff to infectious materials	Mosquito, sporozoite, IBSM	Standard personal protective equipment
Onward malaria transmission	Mosquito, sporozoite, IBSM	Confinement Travel restriction Use of a gametocidal agent qPCR negative at the end of the study Blood donation restrictions Mosquito avoidance In endemic settings, caution with the use of genetically modified parasites or a strain not endemic to the area
Escape of infected mosquitos	Mosquito, sporozoite, IBSM (transmission studies)	Insectary controls

CYP2D6 = cytochrome P-450 isoenzyme 2D6; G6PD = glucose-6-phosphate dehydrogenase; HMP = human malaria parasite; IBSM = induced blood-stage malaria; qPCR = quantitative polymerase chain reaction; *P. vivax* = *Plasmodium vivax*; RBC = red blood cell; VIS = volunteer infection study.

way cannot develop into adult worms,¹⁹ and thus, the risk of harm in such an unlikely circumstance is extremely low.^{20,21}

At our center, HMP banks are tested before release using a process that has undergone regulatory review. This includes testing for microbial contamination and endotoxin. Whole genome sequencing of HMP banks can identify contaminants as well as parasite genotype, clonality, and polymorphisms associated with antimalarial sensitivity.^{22,23}

Transfusion reaction. A transfusion reaction is a risk specific to IBSM VIS. The IBSM process inevitably results in transfusion of a small number of RBCs (up to 1.5×10^9 in the case of *P. vivax* IBSM at our center), which is equivalent to less than 1 mL of whole blood. Subjects are only inoculated with a compatible ABO blood group (and compatible rhesus group in the case of women of childbearing potential). It is remotely possible that the receipt of donor RBCs could precipitate a transfusion reaction or cause development of alloantibodies, which may make future blood transfusion more difficult or result in hemolytic disease of the newborn, if a woman develops alloantibodies because of this process before

becoming pregnant. Acute transfusion reactions are judged to be extremely unlikely because of the very small volume of blood that is administered with the challenge agent and because white cells are removed by leukodepletion during processing. No acute transfusion reactions have been reported in more than 350 subjects who have been infected through IBSM. Nevertheless, subjects are monitored for transfusion reactions after receiving the challenge agent. Subjects are screened before inoculation and at the end of the study for RBC alloantibodies. Two subjects have been reported to develop RBC alloantibodies in the context of IBSM. One was injected with *P. falciparum* 3D7–infected blood group O (RhD)-negative RBCs and developed an anti-E antibody at the end of study blood sampling (Australian and New Zealand Clinical Trial Registry [ANZCTR] ID: ACTRN12614000781640). Adsorption studies confirmed the presence of a true anti-E alloantibody. No irregular anti-RBC antibodies were detected in a sample taken 6 weeks earlier. In addition, the RBCs in the HMP bank were documented to lack the E antigen. Although anti-E antibodies have been implicated in hemolytic transfusion

reactions,^{24,25} it is well established that natural anti-E antibodies may occur without transfusion.^{26,27} A transfusion medicine expert concluded it was most likely that this subject had naturally occurring low-level anti-E alloantibodies and that it was unlikely that these alloantibodies were induced in the study. The second subject participated in a vaccine study in which they were injected with 3×10^7 chemically attenuated asexual whole *P. falciparum* parasites contained within blood group O (RhD)-negative RBCs.²⁸ Parasites were derived from cultures with 5% parasitemia, making the total number of injected RBCs 6×10^8 . This subject was the only of six subjects, who developed antibodies to the minor Rh antigen c. Whether this was related in some other way to preparation of the vaccine is unknown. The authors suggested reducing the number of RBCs per inoculum to decrease the risk of induction of alloantibodies.²⁸ It is not clear why the vaccine induced an antibody response, although there have been no such cases within IBSM studies with *P. vivax* (44 subjects)^{29,30} (ANZCTR ID: ACTRN12617001502325; ANZCTR ID: ACTRN12616000174482; ClinicalTrials.gov ID: NCT02573857) or *Plasmodium malariae* (two subjects),³¹ where means of 6.5×10^8 and 6.8×10^8 RBCs were administered per challenge agent, respectively.

Dose of parasites. The number of parasites injected (i.e., the dose) is a determinant of the starting blood-stage parasitemia. When infection is induced by sporozoite inoculation, the number of infected hepatocytes will determine the starting blood-stage parasitemia,^{32,33} whereas in IBSM, it is the actual number of parasites injected. Mosquito bite VIS typically involve five bites by infectious mosquitos. However, this entails uncertainty regarding the dose a subject receives, which can vary by several thousand sporozoites.^{34–36} Each sporozoite that successfully establishes liver-stage infection results in the production of 25,000–30,000 merozoites destined to invade RBCs. Thus, this variation can significantly impact the ultimate blood-stage inoculation and therefore the time to patency. The use of PCR instead of microscopy reduces prepatency period and enables more prompt diagnosis and treatment of malaria for all forms of VIS.³⁷ For VIS using cryopreserved sporozoites or IBSM, the challenge agent can be better controlled to produce a more reproducible dose (which means less variation in starting parasitemia between subjects) than possible in mosquito bite VIS.^{32,38} This theoretically results in a well-characterized and uniform pattern of growth of parasitemia in vivo. In theory, a single viable parasite is all that is needed, with the duration of the prepatent period depending on the challenge agent dose.

Serious adverse events. Three episodes of cardiac inflammation have been reported in malaria VIS. All three subjects had been infected by mosquito bite in the Netherlands; however, it is not clear whether these cardiac events were related to the malaria infection.^{4,39,40} As a precaution, individuals with significant cardiovascular disease risk factors are excluded at screening in VIS.

Relapse of *P. vivax*. For *P. vivax* mosquito bite VIS, researchers must ensure no liver-stage hypnozoites remain at the end of the study. Although radical cure with primaquine had been considered satisfactory for this purpose, two subjects experienced multiple relapses of *P. vivax* following challenge via mosquito bites despite chloroquine and primaquine treatment.¹ These relapses were discovered to be caused by a previously unrecognized pharmacogenetic effect of polymorphism in the

human cytochrome P-450 isoenzyme 2D6 (CYP2D6).⁴¹ Both subjects were shown to have low activity of CYP2D6 that resulted in them not transforming primaquine into its active metabolite. Therefore, individuals who are poor or intermediate metabolizers of CYP2D6 should not be enrolled in such studies. In addition, individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd) should be excluded from sporozoite-induced *P. vivax* VIS as they should receive primaquine because of the risk of hemolysis. Female heterozygotes may also experience clinically significant hemolysis if moderate deficiency is not excluded by a quantitative assay.⁴² Currently, it is not clear whether poor CYP2D6 activity also affects the activity of primaquine against gametocytes.⁴³ Although tafenoquine (a recently registered 8-aminoquinoline) may represent an alternative to primaquine, tafenoquine can also cause life-threatening hemolysis in individuals with G6PDd. Furthermore, because of its long half-life, the hemolytic effects of tafenoquine cannot be limited by halting treatment as can be practiced with primaquine because tafenoquine is a single-dose regime.⁴⁴ Thus, a higher threshold of G6PD activity in female heterozygotes (> 70%) is required compared with primaquine (> 30%).⁴²

RISKS TO STAFF

Malaria infection. Risks to staff performing VIS include bites from an infected mosquito or inadvertent exposure to infectious material such as by needlestick injury in cryopreserved sporozoite studies and IBSM studies. Staff should use appropriate personal protective equipment and have clear guidelines on how to access a specialist for advice regarding malaria-specific treatment.

RISKS TO THE COMMUNITY

Malaria transmission. Preventing onward transmission of malaria is vital. Malaria VIS have historically been conducted at a small number of research centers in non-endemic countries with high levels of physical containment and health infrastructure (Australia, the Netherlands, the United Kingdom, Germany, and the United States). At our center in Brisbane, Australia, subjects are inoculated at least 8 days before receiving any therapeutic intervention. While *P. vivax* parasites produce gametocytes early in infection,⁴⁵ while *P. falciparum* gametocytes typically appear in the circulation 10 days after inoculation.¹¹ Subjects potentially infectious to mosquitos may be confined indoors to ensure they are not bitten by vector-competent *Anopheles* mosquitos. More commonly, subjects are monitored as outpatients³⁷ and required to adhere to travel restrictions. At our center, subjects are required not to travel in the period between inoculation and curative treatment, to malaria-endemic countries or to northern Australia where *Anopheles farauti* (the major Australian malaria vector) is present.⁴⁶ In Queensland, *An. farauti* mosquitos are not found south of Mackay, 950 km north of Brisbane. No vector-competent *Anopheles* mosquitos are found in Oxford, United Kingdom, or Nijmegen, the Netherlands, although these mosquitos were once endemic in both cities.^{47,48} The vector-competent species *Anopheles quadrimaculatus* is endemic to the east coast of North America^{49,50} and could conceivably result in local transmission in the context of VIS undertaken at centers in Maryland. Climate change models have predicted the expansion of malaria transmission zones in Australia, Europe, and

North America,⁵¹ so researchers must remain vigilant of such changes and alter their practices accordingly.

Mitigation strategies to prevent onward transmission may need to be applied at the end of the malaria VIS, if infection has resulted in the development of gametocytemia. Subjects may require treatment with an appropriate gametocytocidal agent (e.g., primaquine) before exiting the trial. Parasite-negative status can be confirmed using PCR assays that detect both asexual parasites and gametocytes, which are increasingly being used to confirm subjects that are parasite-negative at exit from malaria VIS studies.^{52,53} The 18S qPCR used at our center has a limit of quantitation of 111 parasites/mL. To transmit malaria in a 1-μL mosquito blood meal, the female *Anopheles* mosquito needs to take up one male and one female gametocyte. Thus, it is extremely unlikely that a subject with a negative 18S qPCR result would transmit malaria. Additional gametocyte-specific quantitative reverse transcriptase PCR (qRT-PCR) assays, such as one that targets *pfs25* (the abundant mRNA present in female gametocytes), can also be used to confirm the absence of gametocytes.⁵⁴ Strict enforcement of travel restrictions during the study and qPCR confirmation of parasite negativity before the end of study are essential, particularly if the study entails the deliberate induction of higher gametocyte levels to test transmission blocking interventions.¹¹ Study subjects are not permitted to donate blood until 6 months after the end of the study in Australia, or 3 years in the United States.

Mosquito escape. Escape of a malaria-infected mosquito could result in difficult-to-diagnose and potentially fatal local malaria. Furthermore, the local establishment of an exotic malaria vector would represent a serious breach of biocontainment. Recently updated guidelines from the *American Society of Tropical Medicine and Hygiene* provide strategies to mitigate the risk of arthropod escape.⁵⁵ The primary method is the use of an appropriately secure insectary. Standard operating procedures for mosquito handling and recovery in the event of mosquito escape are required to prevent and rectify mosquito escape.

Genetically modified parasites. Additional regulations, specific to each national jurisdiction, apply to the use and potential release of genetically modified parasite pathogens. This includes genetically modified malaria parasites that have been used in clinical trials^{56,57} (ANZCTR ID: ACTRN12617000824369). The inadvertent release of genetically modified parasites into local malaria vectors could lead to unforeseen or additional negative effects beyond that caused by the release of a wild type organism.

Risks of conducting malaria VIS in endemic settings. The development of malaria VIS in endemic settings represents an important advance in terms of studying infection in naturally exposed populations^{58,59} but imposes additional logistic and ethical considerations. Confinement of study subjects from initial infection to clearance of parasitemia has been one strategy used to mitigate additional risks.⁵⁸ Ideally, the aim would be to ensure the availability of equivalent containment measures and, if possible, qPCR to ensure equivalent biocontainment and subject safety^{60,61} to what is practiced in settings with more advanced health infrastructure. Cryopreserved sporozoite inoculation has been the preferred mode of infection in recent VIS in malaria-endemic settings^{58,62} because of the infrastructure requirements of mosquito bite VIS (insectary maintenance) and IBSM VIS (clean room facilities for malaria challenge agent preparation).

Although measures to mitigate the risk of onward transmission of malaria from gametocytemic subjects to local

mosquitos or the escape of infectious or noninfectious exotic mosquitos do not differ conceptually,⁶³ containment measures should be equally rigorous. As *P. vivax* infection results in the production of transmissible gametocytes early in infection,⁶⁴ confinement of subjects needs to begin at the onset of parasitemia. The unintentional release of a genetically modified parasite or a strain not endemic in the area, especially a parasite associated with artemisinin resistance such as a drug-resistant *P. falciparum* isolate (e.g., a kelch13 mutation such as used in an IBSM VIS conducted at our center; ANZCTR ID: ACTRN12617001394336), would represent a severe breach of biosecurity. Previous exposure to malaria reduces the incidence and severity of adverse events in endemic VIS settings.⁶² However, the spectrum of adverse events in endemic populations is less well studied, and close monitoring is still required.⁶⁵ Practical considerations such as clinical immunity masking the onset of parasitemia and the effect of immunity on the appearance of gametocytemia and time of infectiousness should also be considered.^{11,66}

CONCLUSION

In the context of malaria VIS, it is not enough to react to safety concerns as they occur. Researchers must remain vigilant to potential risks including new risks induced by climate change, changes in regulations, and genetically modified parasites so as to ensure high safety standards vital to the safe conduct of malaria VIS. Furthermore, maintaining an intact reputation of malaria VIS is essential for ongoing support from the scientific community, regulators, the general community, and, most importantly, the subjects.

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REFERENCES

1. Bennett JW et al., 2016. Phase 1/2a trial of *Plasmodium vivax* malaria vaccine candidate VMP001/AS01B in malaria-naïve adults: safety, immunogenicity, and efficacy. *PLoS Negl Trop Dis* 10: e0004423.
2. Bijker EM et al., 2013. Protection against malaria after immunization by chloroquine prophylaxis and sporozoites is mediated by preerythrocytic immunity. *Proc Natl Acad Sci USA* 110: 7862–7867.
3. Spring MD et al., 2009. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. *PLoS One* 4: e5254.
4. Verhage DF, Telgt DS, Bousema JT, Hermesen CC, van Gemert GJ, van der Meer JW, Sauerwein RW, 2005. Clinical outcome of experimental human malaria infection induced by *Plasmodium falciparum*-infected mosquitoes. *Neth J Med* 63: 52–58.
5. McCarthy JS et al., 2016. A phase II pilot trial to evaluate safety and efficacy of ferroquine against early *Plasmodium falciparum* in an induced blood-stage malaria infection study. *Malar J* 15: 469.
6. McCarthy JS, Smith B, Reid M, Berman J, Marquart L, Dobbin C, West L, Read LT, Dow GS, 2019. Blood schizonticidal activity and safety of tafenoquine when administered as chemoprophylaxis to healthy, non-immune participants followed by blood

- stage *Plasmodium falciparum* challenge: a randomized, double-blinded, placebo-controlled phase 1b study. *Clin Infect Dis* 69: 480–486.
7. Nyunt MM, Hendrix CW, Bakshi RP, Kumar N, Shapiro TA, 2009. Phase I/II evaluation of the prophylactic antimalarial activity of pafuramidine in healthy volunteers challenged with *Plasmodium falciparum* sporozoites. *Am J Trop Med Hyg* 80: 528–535.
 8. Bachmann A et al., 2016. Mosquito passage dramatically changes var gene expression in controlled human *Plasmodium falciparum* infections. *PLoS Pathog* 12: e1005538.
 9. Lavstsen T, Magistrado P, Hermesen CC, Salanti A, Jensen AT, Sauerwein R, Hviid L, Theander TG, Staalsoe T, 2005. Expression of *Plasmodium falciparum* erythrocyte membrane protein 1 in experimentally infected humans. *Malar J* 4: 21.
 10. Berna AZ, McCarthy JS, Wang RX, Saliba KJ, Bravo FG, Cassells J, Padovan B, Trowell SC, 2015. Analysis of breath specimens for biomarkers of *Plasmodium falciparum* infection. *J Infect Dis* 212: 1120–1128.
 11. Collins KA et al., 2018. A controlled human malaria infection model enabling evaluation of transmission-blocking interventions. *J Clin Invest* 128: 1551–1562.
 12. Noulon F, Borlon C, Van Den Abbeele J, D'Alessandro U, Erhart A, 2013. 1912–2012: a century of research on *Plasmodium vivax* in vitro culture. *Trends Parasitol* 29: 286–294.
 13. Stanisic DI et al., 2015. Development of cultured *Plasmodium falciparum* blood-stage malaria cell banks for early phase in vivo clinical trial assessment of anti-malaria drugs and vaccines. *Malar J* 14: 143.
 14. Trager W, Jensen JB, 2005. Human malaria parasites in continuous culture. 1976. *J Parasitol* 91: 484–486.
 15. Lim C, Hansen E, DeSimone TM, Moreno Y, Junker K, Bei A, Brugnara C, Buckee CO, Duraisingh MT, 2013. Expansion of host cellular niche can drive adaptation of a zoonotic malaria parasite to humans. *Nat Commun* 4: 1638.
 16. Moon RW, Hall J, Rangkuti F, Ho YS, Almond N, Mitchell GH, Pain A, Holder AA, Blackman MJ, 2013. Adaptation of the genetically tractable malaria pathogen *Plasmodium knowlesi* to continuous culture in human erythrocytes. *Proc Natl Acad Sci USA* 110: 531–536.
 17. Pawliw R et al., 2018. A bioreactor system for the manufacture of a genetically modified *Plasmodium falciparum* blood stage malaria cell bank for use in a clinical trial. *Malar J* 17: 283.
 18. Cervia JS, Wenz B, Ortolano GA, 2007. Leukocyte reduction's role in the attenuation of infection risks among transfusion recipients. *Clin Infect Dis* 45: 1008–1013.
 19. Barbara JAJ, 1983. *Microbiology in Blood Transfusion*. Bristol, United Kingdom: John Bright & Sons Ltd.
 20. Paily KP, Hoti SL, Das PK, 2009. A review of the complexity of biology of lymphatic filarial parasites. *J Parasit Dis* 33: 3–12.
 21. Choudhury N, Murthy PK, Chatterjee RK, Khan MA, Ayyagari A, 2003. Transmission of filarial infection through blood transfusion. *Indian J Pathol Microbiol* 46: 367–370.
 22. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW, 2018. GenBank. *Nucleic Acids Res* 46: D41–D47.
 23. O'Leary NA et al., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res* 44: D733–D745.
 24. Park TS, Kim KU, Jeong WJ, Kim HH, Chang CL, Chung JS, Cho GJ, Lee EY, Son HC, 2003. Acute hemolytic transfusion reactions due to multiple alloantibodies including anti-E, anti-c and anti-Jkb. *J Korean Med Sci* 18: 894–896.
 25. Michalewska B, Ejduk A, Pniowska K, 2005. Acute haemolytic transfusion reaction apparently caused by the 'enzyme-only' anti-E. *Vox Sang* 89: 61.
 26. Kinch RA, Roy RB, 1956. Anti-E, probably naturally occurring. *Can Med Assoc J* 75: 523.
 27. Grove-Rasmussen M, Levine P, 1954. Occurrence of anti-D and anti-E in absence of obvious antigenic stimuli. *Am J Clin Pathol* 24: 145–149.
 28. Stanisic DI et al., 2018. Vaccination with chemically attenuated *Plasmodium falciparum* asexual blood-stage parasites induces parasite-specific cellular immune responses in malaria-naïve volunteers: a pilot study. *BMC Med* 16: 184.
 29. Griffin P et al., 2016. Safety and reproducibility of a clinical trial system using induced blood stage *Plasmodium vivax* infection and its potential as a model to evaluate malaria transmission. *PLoS Negl Trop Dis* 10: e0005139.
 30. McCarthy JS et al., 2013. Experimentally induced blood-stage *Plasmodium vivax* infection in healthy volunteers. *J Infect Dis* 208: 1688–1694.
 31. Woodford J et al., 2019. An experimental human blood stage model for studying *Plasmodium malariae* infection. *J Infect Dis*. Available at: <https://doi.org/10.1093/infdis/jiz102>.
 32. Mordmüller B et al., 2015. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. *Malar J* 14: 117.
 33. Sheehy SH et al., 2013. Optimising controlled human malaria infection studies using cryopreserved *P. falciparum* parasites administered by needle and syringe. *PLoS One* 8: e65960.
 34. Beier JC, Onyango FK, Koros JK, Ramadhan M, Ogwang R, Wirtz RA, Koech DK, Roberts CR, 1991. Quantitation of malaria sporozoites transmitted in vitro during salivation by wild *Afro-tropical Anopheles*. *Med Vet Entomol* 5: 71–79.
 35. Rosenberg R, Wirtz RA, Schneider I, Burge R, 1990. An estimation of the number of malaria sporozoites ejected by a feeding mosquito. *Trans R Soc Trop Med Hyg* 84: 209–212.
 36. Ponnudurai T, Lensen AH, van Gemert GJ, Bolmer MG, Meuwissen JH, 1991. Feeding behaviour and sporozoite ejection by infected *Anopheles stephensi*. *Trans R Soc Trop Med Hyg* 85: 175–180.
 37. Friedman-Klabanoff DJ, Laurens MB, Berry AA, Travassos MA, Adams M, Strauss KA, Shrestha B, Levine MM, Edelman R, Lyke KE, 2019. The controlled human malaria infection experience at the University of Maryland. *Am J Trop Med Hyg* 100: 556–565.
 38. McCarthy JS et al., 2011. A pilot randomised trial of induced blood-stage *Plasmodium falciparum* infections in healthy volunteers for testing efficacy of new antimalarial drugs. *PLoS One* 6: e21914.
 39. van Meer MP, Bastiaens GJ, Boulaksil M, de Mast Q, Gunasekera A, Hoffman SL, Pop G, van der Ven AJ, Sauerwein RW, 2014. Idiopathic acute myocarditis during treatment for controlled human malaria infection: a case report. *Malar J* 13: 38.
 40. Nieman AE, de Mast Q, Roestenberg M, Wiersma J, Pop G, Stalenhoef A, Druihe P, Sauerwein R, van der Ven A, 2009. Cardiac complication after experimental human malaria infection: a case report. *Malar J* 8: 277.
 41. Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, Deye G, Melendez V, Ockenhouse CF, 2013. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. *N Engl J Med* 369: 1381–1382.
 42. Chu CS et al., 2017. Haemolysis in G6PD heterozygous females treated with primaquine for *Plasmodium vivax* malaria: a nested cohort in a trial of radical curative regimens. *PLoS Med* 14: e1002224.
 43. Eziefule AC, Pett H, Grignard L, Opus S, Kiggundu M, Kamya MR, Yeung S, Staedke SG, Bousema T, Drakeley C, 2014. Glucose-6-phosphate dehydrogenase status and risk of hemolysis in *Plasmodium falciparum*-infected African children receiving single-dose primaquine. *Antimicrob Agents Chemother* 58: 4971–4973.
 44. Rueangweerayut R et al., 2017. Hemolytic potential of tafenoquine in female volunteers heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PD Mahidol variant) versus G6PD-normal volunteers. *Am J Trop Med Hyg* 97: 702–711.
 45. Carter R, 1989. Gametocytes. McGregor WWI, Ed. *Malaria*. Edinburgh, United Kingdom: Churchill Livingstone, 253–306.
 46. Northern Territory Government, Department of Health and Families, 2010. *Common Mosquitoes of the Northern Territory. Descriptions of Species, Habitats and Disease Potential*. Medical Entomology Centre for Disease Control. Department of Health and Families. Northern Territory Government, Causarina, NT, Australia.
 47. Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW, 2004. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis* 4: 327–336.
 48. Rios LM, Roxanne Connolly C, 2018. *Featured Creatures: Anopheles quadrimaculatus*. Available at: <http://entnemdept.ufl.edu/>

- creatures/aquatic/Anopheles_quadrimaculatus.htm. Accessed December 20, 2018.
49. Robert LL et al., 2005. *Plasmodium*-infected *Anopheles* mosquitoes collected in Virginia and Maryland following local transmission of *Plasmodium vivax* malaria in Loudoun County, Virginia. *J Am Mosq Control Assoc* 21: 187–193.
 50. Centers for Disease Control and Prevention, 2003. *Local Transmission of Plasmodium vivax Malaria – Palm Beach County, Florida, 2003*. *MMWR Morb Mortal Wkly Rep* 52: 908–911.
 51. Caminade C, Kovats S, Rocklov J, Tompkins AM, Morse AP, Colon-Gonzalez FJ, Stenlund H, Martens P, Lloyd SJ, 2014. Impact of climate change on global malaria distribution. *Proc Natl Acad Sci USA* 111: 3286–3291.
 52. Lyke KE et al., 2017. Attenuated PfSPZ vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. *Proc Natl Acad Sci USA* 114: 2711–2716.
 53. Ishizuka AS et al., 2016. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* 22: 614–623.
 54. Waters AP, McCutchan TF, 1989. Rapid, sensitive diagnosis of malaria based on ribosomal RNA. *Lancet* 1: 1343–1346.
 55. American Committee of Medical Entomology American Society of Tropical Medicine and Hygiene, 2019. Arthropod containment guidelines, version 3.2. *Vector Borne Zoonotic Dis* 19: 152–173.
 56. Butler NS, Schmidt NW, Vaughan AM, Aly AS, Kappe SH, Harty JT, 2011. Superior antimalarial immunity after vaccination with late liver stage-arresting genetically attenuated parasites. *Cell Host Microbe* 9: 451–462.
 57. van Schaijk BC et al., 2014. A genetically attenuated malaria vaccine candidate based on *P. falciparum* b9/sharp gene-deficient sporozoites. *Elife* 3: e03582.
 58. Hodgson SH et al., 2015. Lessons learnt from the first controlled human malaria infection study conducted in Nairobi, Kenya. *Malar J* 14: 182.
 59. Shekalaghe S et al., 2014. Controlled human malaria infection of Tanzanians by intradermal injection of aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites. *Am J Trop Med Hyg* 91: 471–480.
 60. Murphy SC et al., 2012. Real-time quantitative reverse transcription PCR for monitoring of blood-stage *Plasmodium falciparum* infections in malaria human challenge trials. *Am J Trop Med Hyg* 86: 383–394.
 61. Kamau E, Alemayehu S, Feghali KC, Komisar J, Regules J, Cowden J, Ockenhouse CF, 2014. Measurement of parasitological data by quantitative real-time PCR from controlled human malaria infection trials at the Walter Reed Army Institute of Research. *Malar J* 13: 288.
 62. Achan J et al., 2019. Serologic markers of previous malaria exposure and functional antibodies inhibiting parasite growth are associated with parasite kinetics following a *Plasmodium falciparum* controlled human infection. *Clin Infect Dis*. Available at: <https://doi.org/10.1093/cid/ciz740>.
 63. Juliano SA, Lounibos LP, 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecol Lett* 8: 558–574.
 64. Carter R, Graves PM, 1988. Gametocytes. Wernsdorfer WH, McGregor I, Eds. *Malaria: Principles and Practices of Malariology*. London, United Kingdom: Churchill Livingstone, 253–306.
 65. Roestenberg M, Mordmuller B, Ockenhouse C, Mo A, Yazdanbakhsh M, Kremsner PG, 2017. The frontline of controlled human malaria infections: a report from the controlled human infection models workshop in Leiden University Medical Centre 5 May 2016. *Vaccine* 35: 7065–7069.
 66. Meibalan E, Marti M, 2017. Biology of malaria transmission. *Cold Spring Harb Perspect Med* 7: a025452.
 67. Roestenberg M, de Vlas SJ, Nieman AE, Sauerwein RW, Hermesen CC, 2012. Efficacy of preerythrocytic and blood-stage malaria vaccines can be assessed in small sporozoite challenge trials in human volunteers. *J Infect Dis* 206: 319–323.