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Review

Utilizing direct skin feeding assays for development of vaccines that interrupt malaria transmission: A systematic review of methods and case study

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ABSTRACT

Shifting the malaria priorities from a paradigm of control and elimination to a goal of global eradication calls for renewed attention to the interruption of malaria transmission. Sustained progress toward eradication will require both improved understanding of infectious reservoirs and efficient development of novel transmission-blocking interventions, such as rapidly acting and highly efficacious therapeutics and vaccines. Here, we review the direct skin feeding assay (DSF), which has been proposed as a valuable tool for measuring the *in natura* transmission of malaria parasites from human hosts to mosquito vectors across heterogeneous populations. To capture the methodological breadth of this assay's use, we first systematically review and qualitatively synthesize previously published investigations using DSFs to study malaria transmission in humans. Then, using a recent Phase 1 trial in Mali of the Pfs25H-EPA/Alhydrogel® vaccine candidate (NCT01867463) designed to interrupt *Plasmodium falciparum* transmission as a case study, we describe the potential opportunities and current limitations of utilizing the endpoints measured by DSF in making early clinical decisions for individually randomized transmission-interrupting intervention candidates. Using simulations based on the data collected in the clinical trial, we demonstrate that the capacity of the DSF to serve as an evaluative tool is limited by the statistical power constraints of the "effective sample size" (i.e. the number of subjects that are capable of transmitting at the time of feeding). Altogether, our findings suggest DSFs have great potential utility for assessing the public health impacts of emerging antimalarial tools, but additional research is needed to address issues of scalability and to establish correlation with community-wide clinical endpoints as well as complementary in vitro measures, such as standard membrane feeding assays.

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1. Introduction

Progress toward global malaria eradication will be expedited through the careful integration of established malaria elimination strategies with novel transmission-interrupting interventions operating across the host, parasite, and vector levels [1,2]. As highly cost-effective public health tools with lasting effects on populations, "vaccines that interrupt malaria transmission" (VIMTs) are likely to be central to future elimination efforts [1]. VIMTs include classical transmission-blocking vaccines that can arrest the sporogonic development of the parasite inside the mosquito as well as highly effective pre-erythrocytic vaccines and blood-stage vaccines that may decrease the size of the infectious reservoir by reducing gametocyte carriage. VIMTs in development will achieve the "minimally acceptable target" of expected efficacy if the vaccine, in combination with existing malaria elimination tools, maintains R_e (i.e. the number of subsequent infections arising from each infected human in a population made up of both susceptible and non-susceptible hosts) below one for at least one year in a previously malaria-endemic region [1]. Although the concept of transmission-interrupting interventions is not new (e.g. indoor residual spraying is a highly effective method for decreasing transmission by shortening mosquito lifespans and reducing population densities), the individual-level randomization that is possible with VIMT candidates creates demand for new analytical frameworks. Barring completion of costly and complex cluster randomized controlled trials that evaluate measures such as the entomological inoculation rate or clinical incidence of malaria within communities [3], robust functional assays that provide potential surrogate measures of individuals' infectivity to mosquitoes have been proposed as the best available approaches for evaluating early clinical Go/No Go decisions for VIMT candidates in the research pipeline.

At present, standard membrane feeding assays (SMFAs), which have been qualified according to the International Conference on Harmonisation guidelines, are considered the gold standard for evaluating the transmission-blocking potential of test antibodies *ex vivo* [4,5]. The SMFAs, which evaluate the functionality of test antibodies by offering cultured *Plasmodium* gametocytes combined with fractionated serum or purified immunoglobulin to laboratory-reared, uninfected mosquitoes through an artificial membrane, have been used to rank the activity of transmission-blocking vaccine candidates [4,6,7]. Whereas SMFAs specifically measure antibody activity in relation to reduction in gametocyte transmission to mosquitoes, broadly defined VIMTs may also incorporate pre-erythrocytic or blood-stage vaccines that require study of human infection. Direct membrane and skin feeding assays can enable investigators to evaluate both reductions in malaria infections and the resulting interruption in transmission of parasites from human blood to mosquitoes. The direct membrane feeding assay (DMFA) is similar in design to the SMFA except that gametocytes are derived from the venous blood of infected individuals and better reflect the diversity of field populations [8]. First described by Muirhead-Thomson in 1957 as a method to test "the malarial infectivity of the human subjects in a direct manner," the direct skin feeding assay (DSF) measures the prevalence and magnitude of infection in laboratory-reared mosquitoes that are contained in cups and allowed to feed directly on the skin of human volunteers [9].

While membrane feeding assays will continue to serve as valuable and well-controlled tools for assessing the blockade of transmission, methods that examine host-vector interactions through direct skin feeds may offer greater scope for investigating the activity of candidate VIMTs on *in natura* transmission. Overall, there is a high concordance between the direct membrane and direct skin feeding methods, and DSFs offer additional biological advantages. An analysis of 241 paired transmission experiments demonstrated a significant positive correlation (Spearman's rho of 0.36, $p < 0.0001$) between the proportion of mosquitoes infected using DMFAs versus DSFs [8]. However, DSFs offer 2-fold higher efficiency in terms of the proportion mosquitoes infected than DMFAs and are also more robust to human error [8,10,11]. In DMFAs, failure to maintain blood samples at a constant temperature of 37 °C could lead to the premature activation of gametocytes and interfere with gametocyte infectiousness and successful parasitic reproduction [12,13]. Further, DSFs better mimic *in vivo* feeding conditions than DMFAs. By design, membrane feeding assays eliminate differences in host attractiveness to mosquitoes and are also incapable of simulating the immune response and gametocyte densities and dynamics that occur in host microvasculature [14].

Table 1
Search strategy for the literature-based systematic review.

Database	Search terms
PubMed	(direct feed* [tiab] or direct skin feed* [tiab] or feeding assay [tiab] or feed assay [tiab]) & (Vaccine [MeSH] or Malaria Vaccines [MeSH] or Malaria [MesH] or Malaria, falciparum [MeSH] or malaria [tiab] or Culicidae [MeSH] or mosquito* [tiab] or oocysts [MesH] or oocyst* [tiab])
Web of Science	((("direct feed*" OR "direct skin feed*" OR "feed assay" OR "feeding assay") AND (vaccine* OR malaria OR falciparum OR Culicidae OR mosquito* OR oocyst*)))
EMBASE	'direct feed' OR 'direct feeding' OR 'direct skin feed' OR 'direct skin feeding' OR 'feed assay' OR 'feeding assay' AND ('vaccine'/exp OR 'vaccine' OR vaccine* OR 'malaria vaccine'/exp OR 'malaria vaccine' OR falciparum OR 'culicidae' OR 'culicidae'/exp OR culicidae OR mosquito* OR oocyst*) AND ([article]/lim OR [article in press]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim OR [review]/lim OR [short survey]/lim)

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