Design and Testing of a Novel, Protective Human-Baited Tent Trap for the Collection of Anthropophilic Disease Vectors

Author(s): Benjamin J. Krajacich , Jeremiah R. Slade , Robert T. Mulligan , Brendan Labrecque , Kevin C. Kobylinski , Meg Gray , Wojtek S. Kuklinski , Timothy A. Burton , Jonathan A. Seaman , Massamba Sylla , and Brian D. Foy

Source: Journal of Medical Entomology, 51(1):253-263. 2014.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/ME13090

Design and Testing of a Novel, Protective Human-Baited Tent Trap for the Collection of Anthropophilic Disease Vectors

BENJAMIN J. KRAJACICH,[1,2] JEREMIAH R. SLADE,[3] ROBERT T. MULLIGAN,[3] BRENDAN LABRECQUE,[3] KEVIN C. KOBYLINSKI,[1] MEG GRAY,[1] WOJTEK S. KUKLINSKI,[1] TIMOTHY A. BURTON,[1] JONATHAN A. SEAMAN,[1] MASSAMBA SYLLA,[1] AND BRIAN D. FOY[1,2]

J. Med. Entomol. 51(1): 253–263 (2014); DOI: http://dx.doi.org/10.1603/ME13090

ABSTRACT Currently, there exists a deficit of safe, active trapping methods for the collection of host-seeking *Anopheles* and other disease-causing arthropod vectors. The gold-standard approach for mosquito collection is that of human landing catch (HLC), in which an individual exposes bare skin to possibly infected vectors. Here, we present the development of a new method for mosquito collection, the Infoscitex tent, which uses modern tent materials coupled with a novel trap design. This provides an efficacious, a non-labor-intensive, and a safe method for vector collection. In these initial studies, we found it collected an average of 27.7 *Anopheles gambiae* s.l. per trap per night in rural villages in southeastern Senegal, and 43.8 *Culex* group V per trap per night in the semiurban town of Kedougou, Senegal. In direct comparisons with HLC, the tent was not statistically different for collection of *Culex quinquefasciatus* in crepuscular sampling, but was significantly less efficacious at trapping the highly motile dusk-biter *Aedes aegypti*. These studies suggest that the Infoscitex tent is a viable and safe alternative to HLC for *Anopheles* and *Culex* sampling in areas of high vector-borne disease infection risk.

KEY WORDS mosquito, malaria, vector, trap, tent

The development of an effective and a safe sampling method for the collection of host-seeking anthropophagic mosquito vectors has long been a goal for medical entomologists. Human landing catches (HLCs) are considered the gold standard for sampling host-seeking anthropophagic mosquito populations and estimating the human biting rate (HBR), which is needed to measure the entomological inoculation rate. HLC consists of trained collectors luring host-seeking mosquitoes from the environment with his or her own cocktail of volatiles, gases, body heat, and humidity, and collecting the vectors that land on and attempt to bite an exposed part of their body, usually their legs. There are inherent risks of contracting mosquito-borne pathogens with this technique, as mosquitoes transmit nematodes, arboviruses, and *Plasmodium* through probing alone, before imbibing any blood (Ewert and Ho 1967, Ho and Ewert 1967, Medica and Sinnis 2005, Styer et al. 2007), and it is very difficult to only capture landing mosquitoes over a sampling interval without having any of them probe. HLC for arbovirus mosquito vectors can put nonimmune collectors at particular risk because only supportive therapies are available for arboviral diseases. In malaria vector research, curative and prophylaxis drug regimens for *Plasmodium* infections lower the risk to the collectors, and so HLCs are used more routinely, but it is not widely acknowledged that *Anopheles* can also transmit a variety of arboviruses in many areas of the world, such as O'nyong-nyong virus and Bwamba virus (Williams et al. 1965; Lutwama et al. 1999, 2002). Because of these risks, some ethical review boards have deemed HLCs unethical and will not approve them, while others have put constraints on how they are conducted, including requiring all collectors to take malaria prophylaxis medication and to undergo routine blood smear examinations during their work. The World Health Organization recommends not performing HLC in malaria vector research when safer methods are available to estimate the HBR (World Health Organization 2003).

The alternatives to HLC are using various designs of nets or bed nets that both surround and protect the human bait while passively or actively capturing the host-seeking mosquitoes that come to bite. These human-occupied net traps have been used since the early 1900s (Silver 2008). Passive or semipassive trap designs have been the most common, whereby a person rests or sleeps under a bed net, while host-seeking

[1] Department of Microbiology, Immunology and Pathology, Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523.

[2] Corresponding author, e-mail: bkrajacich@gmail.com; brian.foy@colostate.edu.

[3] Force Protection & Sustainment, National Security Solutions, Infoscitex Corporation, 303 Bear Hill Road, Waltham, MA 02451.

mosquitoes pass through a window, through a funnel, or under a gap of an outer entrapment net (Reid 1961, Silver 2008). Passive designs may have a disadvantage in that some species are highly capable of exiting even small gaps and funnel holes from which they entered the trap (Charlwood et al. 1986, Darbro and Harrington 2006). The Mbita trap is a passive trap that uses a funnel trap attached on top of the bed net (Mathenge et al. 2002). It has been successful in estimating HBR in some studies (Mathenge et al. 2004, 2005), but unsuccessful in other studies (Laganier et al. 2003, Braimah et al. 2005). In semipassive designs, the collector drops a flap over the open window or drops the outer net to close the gap and entrap the host-seeking mosquitoes after the collecting interval is completed (Silver 2008). Most of these passive and semipassive designs require the collectors to then spend their time aspirating mosquitoes from the relatively large holding chamber, which can be laborious and can lead to risk of being bitten (Govella et al. 2009). Trap collection counts can also be substantially reduced when mosquitoes need to navigate through a window, gap, or slit in the entrapment net (Le Goff et al. 1997). There has been recent success using the Ifakara tent designs for purely passive mosquito collection (Govella et al. 2009, 2011; Wong et al. 2013). These designs can be quite effective, meeting or exceeding capture levels to that of HLC for *Anopheles* species, although *Culex* spp. are caught at a decreased rate (Wong et al. 2013).

Active trapping system alternatives to HLC most often attach a fan trap to the entrapment net, and usually augment the attractiveness with a light. Charlwood et al. attached an inverted CDC light trap over the outer entrapment net, and the design was successful in capturing host-seeking *Anopheles farauti* (Charlwood et al. 1986). The odor-baited entry trap (OBET) was designed for anemotactic behavioral studies in the laboratory, but modified for successful field capture of African *Anopheles* malaria vectors (Constantini et al. 1993). The OBET separates the host-holding tent from the capture device, and connects the two with a hose through which a fan system blows odors from the host-holding tent through the capture device. In Senegal, the OBET accurately reflected outdoor HLC, but not indoor HLC (Dia et al. 2005). Mutero et al. used a homemade updraft trap suspended over a bed net to successfully capture host-seeking African malaria vectors (Mutero and Birley 1987). Many investigators now use a similar design, but for consistency, most use commercially purchased Center for Disease Control and Prevention (CDC) mini-light traps (LTC) hung next to the feet of a human resting under a bed net (Lines et al. 1991, Ndiath et al. 2011, Overgaard et al. 2012). Several investigators have observed no differences in *Anopheles* capture rates if long-lasting permethrin-treated bed nets are used in LTC rather than untreated bed nets, thus improving the safety for the collector (Magbity et al. 2002, Fornadel et al. 2010, Govella et al. 2011). In some studies, LTC accurately reflected the species composition, capture rates, or vector bionomics of the HLC (Mathenge et al. 2004, Govella et al. 2009), whereas in other studies, consistent associations failed (Overgaard et al. 2012). When LTC accurately reflects HLC, a conversion factor is typically needed to adjust the raw numbers because the LTC tends to underestimate the number of mosquitoes captured by HLC (Overgaard et al. 2012).

Missing so far from the panoply of trapping systems is a standardized active trapping system for use with human hosts that accurately samples certain host-seeking vectors, that is simple to use, that is easily adjustable to attract different vectors, that is comfortable for the human bait, and that makes use of modern camping tent technology. Modern camping tents have been developed for decades so they are now lightweight and contained for easy transport by a single user. They are also efficiently designed to require minimal time to set up and tear down, and they can be extremely rugged and protective in diverse weather situations. Finally, they protect the human inside from the majority of biting vectors while still maintaining airflow by using micromesh fabric panels, thus they offer both comfort and protection for the user. Here we describe the development and initial testing of a modern collection technique using the benefits of standardized tent technology coupled with a novel, safe active trapping system.

Materials and Methods

Tent Trap Design and Construction. The tent trap was designed by engineers at the Infoscitex Corp. (IST; Waltham, MA) by modifying a three-person Losi tent (Nemo Equipment Inc., Dover, NH). The final design consisted of a standard rectangle-shaped footprint measuring 91″ by 79″ (50 sq. ft.) with zippered doors on the two longer sides (Fig. 1A). The tent trap walls consist of lightweight breathable 70 denier nylon ripstop fabric. The floor was made of 70D waterproof nylon taffeta fabric. The rain fly cover is made out of 70D waterproof nylon rip stop fabric. Air venting material was composed of 2010 "no-see-um" nylon mesh with grid sizes approximately $\approx$0.25 mm^2 and situated at the top of the fabric wall panels on all four sides. The supports are made from DAC Featherlite anodized aluminum poles. Suction ports for vector capture were placed immediately above the air venting panels on the four walls to aspirate vectors following the source of the odor plume. Plastic suction ports had a 20.5-cm-wide and 2.5-cm-high intake opening, and were designed with a lip that faced down into the odor plume (Fig. 2B). Air intake tubes connect the suction ports to the manifold integrated into the center top of the tent and run along the outer top of the tent (Fig. 1A); the long axis tubes were 95.5 cm in length and the short axis tubes 52 cm. The manifold was designed to fit into gasketed fabric on the tent ceiling, and only be open to incoming vectors when the vector trap was fully installed below it. The cone-shaped vector trap (Fig. 3A–F) with $\approx$0.8-mm steel mesh was designed to be screwed into the manifold, to open when the fan was closed underneath it, and to close when the fan attachment was opened underneath (Fig. 3A). The fan

Fig. 1. Picture of tent at an angle with rain fly off showing ports attached to center manifold (A) and with rain fly on (B). Arrows indicate vector entry point at ports (A) or through gaps in rain fly leading to ports (B).

pulls vectors into the trap from the bottom of the manifold-trap apparatus. The fan is powered by 12-V sealed lead acid gel cell batteries (108 Watt hours) and controlled by a power conditioning and control system developed by IST. The tent trap was designed to work with or without a rain fly to facilitate its use in all climates and weather conditions. The rain fly covers the entire tent and has two overhanging gaps on each long side and a one ground gap on each short side to allow vectors to fly into the space between the fly and the tent (Fig. 1B, indicated by arrows).

Mosquitoes. *Aedes aegypti* Chetumal (L.) and *Anopheles gambiae* sensu stricto (s.s.) (Giles) were reared at 28 ± 2°C, 80% humidity, under a photoperiod of 14:10 (L:D) h. *Aedes* larvae were reared in 28-liter containers filled with ≈15 liters of tap water and fed a diet of ground Tetramin fish food mixed with ground mouse food. *Culex quinquefasciatus* (Say) larvae were reared in a similar fashion. *Anopheles* larvae were reared in 44-liter bins with 15 liters of tap water, and fed a diet of ground Tetramin fish food. All adult mosquitoes were provided with water and raisins or 10% sucrose as a sugar source ad libitum. Adult mosquitoes were separated by aspiration into release containers at least 24 h before testing and provided with sugar and water to imbibe freely. Twelve hours before testing, the sugar source was removed. They were kept in a separated, humidified, and temperature-controlled insectary on a 14:10 (L:D) h cycle until used in experiments.

Fig. 2. Overview of tent suction port showing: top-down view (A), installed port showing opening design (B).

Assaying the Prototype Tent Materials. A spatial repellency assay was used with swatches of the tent trap construction material to first determine whether the tent trap construction materials were repellent or attractive to *Ae. aegypti*, Chetumal strain. The assay chamber was similar in design to the high-throughput screening chamber described by Grieco et al. (2005), except it was modified to increase the proportion of mosquitoes responding in the assay (moving from the center cylinder to either end of the chamber) (Grieco et al. 2005). This was done by introducing human breath from the researcher that was gently blown into each end of the cylinder from a bifurcating tube originating near the operator's mouth, as well as from body heat emanating from the researcher's hands being placed over each end cap, for the duration of each test. The assays were performed in a fume hood, with temperature maintained between 21 and 27°C and 20–22% RH. For each test, a fabric swatch was placed in the holding chamber of one end, and nothing was placed in the opposite (control) end; the fabric was moved to the opposite side on each consecutive test and six replicates were performed for each fabric swatch. The spatial activity index was calculated from 20 female mosquitoes per replicate, assaying movement from the center chamber into either end of the assay chamber.

Prototype Tent Trap Efficacy. To assess the mosquito-trapping ability of the initially conceived tent design, a prototype tent trap was deployed in a 2.5- by 4.6-m insectary at the Colorado State University for capturing colonized mosquito vectors released into the room with or without a human in the tent. Note: the prototype design was different from the final design in that it consisted of air venting nylon mesh on the ceiling of the tent, in addition to the side wall panels. In addition, the vector capture trap was not yet designed and installed, and instead a modified capture net was fitted over the fan housing. Twenty, 5–7 d postemergence *Ae. aegypti* (Chetumal strain) and *An. gambiae* (G3 strain) females were used in each test. Insectary humidity was maintained between 70 and 85% for each test and temperature was maintained between 27 and 31°C. Replicate tests were performed sequentially in random order determined by flip of a coin. Mosquitoes were released from a cage in the back of the room at the start of each test, following the tent fans being activated and the tent door being sealed (with or without human bait inside). After releasing mosquitoes, the operator immediately left the room and allowed the mosquitoes to host seek in the room for 20 min during each test with the lights on. Following each test, the operator reentered the room

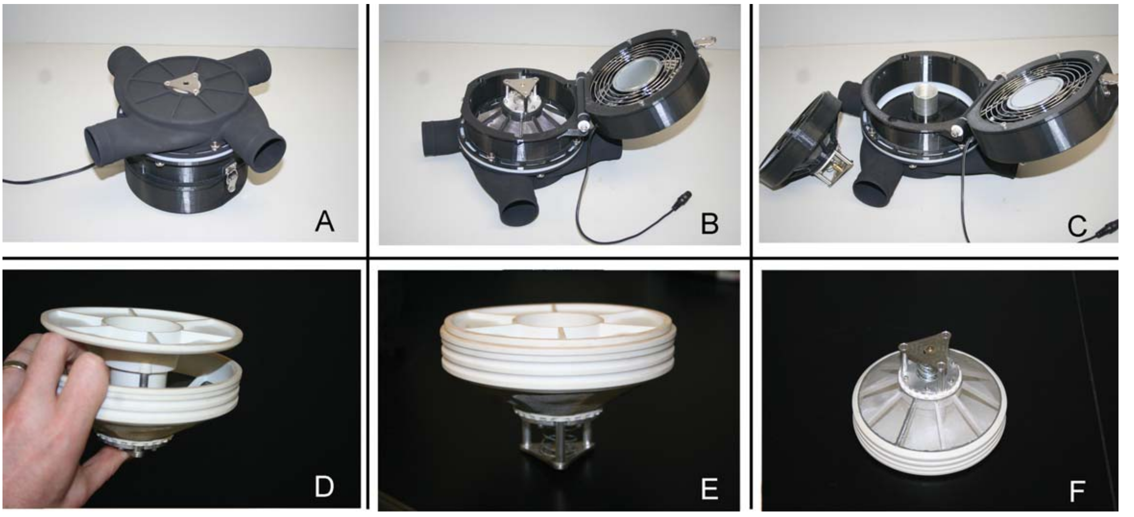

Fig. 3. (A–F). Pictures of whole manifold (A), vector trap screwed in manifold with fan attachment open (flipped upside-down) (B), manifold with vector trap removed (flipped upside-down) (C), vector trap open from side (D), closed collection chamber from above (E), and below (F). Note: D–F are the newer version of the collection chamber. This design added the spokes to limit vector movement, and used white plastic for easier vector viewing/removal.

and used a back pack aspirator to aspirate any uncaught mosquitoes from the room. The insectary was vented with a fan blowing from the insectary into the hallway for 2–5 min between each test.

Computational Fluid Dynamics. Following fabric and prototype testing, a powerful computational fluid dynamics (CFD) tool named SolidWorks Flow Simulation (SolidWorks Corp.) was used at IST to model airflow patterns on the finalized tent trap design and its critical components. This design effort sought to maximize the output of human-generated attractants from the tent and to maximize the effect of the suction trap. Parameters that were modeled included: 1) inlet and outlet port locations, 2) inlet and outlet port size and shape, 3) fan mass flow, and 4) inlet tube diameter. This tool allowed for design down-selection for rapid product development, and enabled an evaluation of numerous tent traps in a virtual environment that would otherwise be too costly to achieve. In parallel with the design effort, various trap containers, lightweight fans, solar charging panels, and other hardware were identified for integration.

Second-Generation Tent Trap Insectary Testing. A CFD reengineered tent trap was deployed in a larger 4.5- by 3.6-m insectary at the Colorado State University for comparative trials against HLC using colonized mosquito vectors released into the insectary. The fabric window panels below each port were rolled down to expose a 2″ gap of mesh fabric for bait-scented air exhaust (see Results). For each trial, 40, 5–7 d postemergence female mosquitoes (*Ae. aegypti*, HWE strain; *An. gambiae*, G3 strain; or *Cx. quinquefasciatus*, Wadsworth strain) that had been sugar-starved, but not water-deprived, for 12–16 h before were released into one corner of the insectary. One researcher stayed in the operational tent trap while the other performed an HLC while seated in a chair in one corner of the insectary. The researcher performing the HLC was fully covered with clothing, except for his or her left foot and leg, which was exposed to the knee. Any mosquitoes landing on the exposed skin were aspirated by a handheld electric aspirator. To control for differential attractiveness, each trial always consisted of paired successive experiments whereby the two researchers switched places in a Latin squares rotational design. Following each 30-min test, the room was aspirated for any non-host-seeking or uncaught mosquitoes. The insectary was opened and air evacuated into the hallway with a fan to remove conflicting volatiles between experiments.

Field Tests in Senegal. Preliminary sampling with the tent trap was performed in the town of Kedougou and the outlying villages of Damboucoye and Nathia in southeastern Senegal. The anthropophilic mosquito vectors *Ae. aegypti*, *Aedes vittatus*, *An. gambiae*, and *Anopheles funestus* are prevalent in this area during the rainy season, and the region is endemic for the transmission of malaria parasites and many arboviruses (Monlun et al. 1993). Comparative testing between the tent trap (without protective rain fly) and HLC was performed by two researchers placed 10–15 m apart in a semiurban portion of Kedougou during evening crepuscular periods (18:00–20:00) when *Ae. aegypti* and *Ae. vittatus* are actively host seeking. The property was flanked by thatched roof huts with open eaves, and several concrete housing structures. Sampling of human overnight host-seeking vectors was performed in Kedougou and the two outlying villages from 22:00 to 6:00. Due to time and resource limitations, HLCs were not performed in Damboucoye and Nathia. All captured mosquitoes were frozen until dead in a −20°C freezer, and then identified to species or possible lowest taxa group using taxonomic keys

(Pecor, Diagne et al. 1994). Species discrimination between members of the *An. gambiae* s.l. complex was done via multiplex polymerase chain reaction (PCR) (Wilkins et al. 2006). Species discrimination for mosquitoes keyed to *Culex* Group V (CGV) was done on a subsample of mosquitoes in a separate multiplex PCR (Smith and Fonseca 2004).

Statistical Analysis. Comparisons of proportions captured by the prototype tent were performed with contingency tables and a two-tailed Fisher exact test. To assess difference among the trapping methods (tent vs. HLC), two-tailed paired *t*-tests were performed with $\log(x + 1)$ transformed data. Data were analyzed with GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, www.graphpad.com). Tests during the crepuscular biting period from Kedougou, Senegal, were analyzed using the Wilcoxon matched pairs test due to the unknown level of normality and small sample sizes of this portion of the data set.

Human Subjects. All HLC experiments were performed by trained volunteers who had read and signed required informed consent documentation. HLCs were performed following human subjects' research protocols approved by the Institutional Review Board at the Colorado State University, and in compliance with the Helsinki Declaration. HLC in Senegal was performed as described for laboratory testing, but with the addition of a head net. Collectors were provided with atovaquone + proguanil for the duration of the testing.

Results

System Design and Prototype Testing. The overall system design was to mount a battery-powered fan in the center tent ceiling that would blow air on the human laying inside, and push their volatiles and odors primarily out of the venting patches on each side of the tent. This creates odor plumes that would attract flying insect vectors from any of the four sides. Simultaneously, air intake suction ports leading to the vector trap housed immediately above the fan would be positioned over the venting patches to actively capture vectors flying toward the odor source. With proper engineering, it was hypothesized that a portion of the odor plume would be recirculated by the fan trap while some would exit the venting patches and create an odor plume. Air intake tubes connect the suction ports to the manifold integrated into the center top of the tent. Spatial assays confirmed that the tent materials were not significantly attractive, nor repellent, to *Ae. aegypti* mosquitoes (Supp. Table 1 [online only]). Approximately half of the released *Ae. aegypti* mosquitoes were captured by the prototype tent in initial testing, but the proportions were irrespective of whether the tent was occupied by a person ($P = 0.24$) (Table 1). The human-occupied prototype tent trap successfully captured more colonized *An. gambiae* mosquitoes than an unoccupied prototype tent ($P = 0.01$) (Table 1). It was observed by the tent operator that the open mesh tent ceiling of the prototype caused many mosquitoes to host seek on the top rather than being directed to the suction ports over the side walls where they could be captured. Likewise, it was noted that the modified capture net fitted over the fan housing on the prototype seemed to stifle proper air circulation, and the quality of the captured mosquitoes was poor because they had to pass through the fan. On reengineering, the ceiling mesh was replaced with tent fabric and the vector trap was designed to fit in-between the manifold and the fan, so that the fan pulled specimens into the trap and they would not pass through the fan blade in a fashion similar to CDC light traps.

Table 1. Proportions of mosquitoes captured by prototype tent

Species tested	Tent status	% captured [95% CI]
Aedes aegypti	Human-occupied	58 [44.7, 71.3]
	Empty	46 [32.5, 59.6]
Anopheles gambiae	Human-occupied	38 [24.3, 51.1]
	Empty	14 [4.3, 23.7]

Computational Fluid Dynamics Modeling. Initial modeling efforts focused on the diameter of the air intake tubes and its impact on airflow velocity and volumetric flow rates. Preliminary design of the lengths of these tubes measured 20″ for the front and back tubes, and 40″ for the side tubes. The tube diameters investigated were 3″, 2″, 1.5″, and 1″. For simplicity, the tubes were all assumed to be straight, and the inlet/suction port was assumed to be conical. Several reference planes were inserted along the flow paths in the model to aid in the postprocessing of the flow results (Supp. Fig. 1A [online only]). Supp. Fig. 1B,C (online only) show typical air velocity intensity images for two different tube diameters. Supp. Fig. 2 (online only) graphs the velocity of the air at the different reference plane locations detailed in Supp. Fig. 1 (online only). As expected, the 1.5″ and 1.0″ (in diameter) tubes produce significantly faster air flow than the 2.0″ and 3.0″ (in diameter) tubes. The tent trap was designed to mimic the performance of the CDC updraft trap that had a reported air velocity between 19 and 39 in/s at the trap opening (Hoel et al. 2009). The simulated velocities at the intake entrance for all four tube diameters are <52 in/s, but the drastic increase in air velocity seen as the air enters the 1.0″ and 1.5″ (in diameter) tubes could potentially cause damage to vector specimens. In addition to the air velocity, the volumetric flow rate in cubic feet per minute for each tube diameter and length was calculated (Supp. Table 3 [online only]). These data showed relatively small reductions in flow rates between the two lengths of tube for a given diameter, but much larger (≈38%) reductions in flow rates as tube diameter decreased from 3″ to 1″. Based on these results, the diameter of the tubes was set at 2.0″ to achieve a balance between airflow velocity and volumetric flow rate. This dimension also provided a gap between the tubes and the rain fly, which helped to reduce the chance of water leaking through the rain fly material.

Table 2. Airspeed at trap intake of IST tent trap against other vector trapping methods

Test parameter	IST tent	Standard CDC (under lid)	Inverted CDC (no intake bowl)	CDC updraft (with intake bowl)	CDC updraft (bowl and ball)	MMX trap
Airspeed at edge of trap intake (m/s)^2	0.35–0.65	1.5–1.7[a]	2.2–2.6[a]	0.5–1.0[a]	1.7–1.9[a]	4.2–4.6[a]

[a] Hoel et al. (2009).

The relationship between the size and location of the exhaust air windows and the quantity of bait-laden air that was recirculated back into the suction ports was also examined using CFD. The overall goal was to determine which exhaust-window and suction-port configuration would give some amount of recirculated air so that there is bait-scented air at the collection port, drawing the vectors in, but still a sufficient amount of bait-scented air being blown into the area surrounding the tent to create an odor plume along which vectors can orient and follow into the suction port. It was assumed that all four mesh windows, one on each side of the tent, were the same size and shape and could be covered with a set of five patches of rip-stop tent fabric; three rectangular patches in the center (top, middle, and bottom) and one triangular patch on either side. This configuration provided more control over the location and velocity at which the exhaust plumes exit the tent. In the model, each of these regions could be made permeable (mesh covered) or impermeable (tent fabric covered) as needed. The actual air permeability of the mesh windows located below each inlet port and the rip-stop fabric that was selected for the main body of the tent were determined based on empirical measurements of the pressure drop across these materials, and this was incorporated into the models. The suction ports were also modeled to create a lower profile that would make them easier to integrate with the tent structure. The results from three of these simulations are shown in Supp. Table 2 [online only] and Fig. 4. For the first simulation (Fig. 4A and B; F02–003), having only the top-center patch open created conditions where almost all of the bait-scented air coming from the tent was immediately recirculated back into the tent via the inlet port. For the simulations where all of the patches were open (Figs. 4C and D; F02–002) or where only the center patches were open (Figs. 4E and F; F02–004), a more balanced scenario appeared where some of the exhaust air is recirculated but some is expelled from each side of the tent to generate an odor plume. However, the air recirculation percentages for the upper and lower intake ports were different for the latter two simulations (Supp. Table 2 [online only]). Based on these simulations, it was determined that a balance of air recirculation and odor plume generation could be best achieved at each port by reconfiguring and manually manipulating the window panels while holding the fan speed steady.

Second-Generation Tent Trap Modification and Insectary Testing. The CFD redesigned tent trap was employed in laboratory tests whereby a 2″ gap of mesh was exposed in the center panel below each suction port to exhaust bait-scented air and maximize catch rates. The average air intake velocity was measured with an anemometer having a 1″ (in diameter) impeller at the center of the lower and upper suction ports, and shown to be consistent with measurements from six other vectors traps (Table 2) (Hoel et al. 2009). The recirculation of air exiting the mesh exhaust windows in this final design was also visibly confirmed using CO_2 fumes (generated by dropping dry ice into beakers of water) emanating from within the tent. With the redesigned trap, mosquito attraction to nonport locations was not observed. Comparison between HLC and the tent across rotational trap sessions in the laboratory showed that the tent trap caught, on average, 58.7% of colony *An. gambiae* caught by HLC (P = 0.0068) and 12.4% of colony *Ae. aegypti* caught by HLC (P = 0.0085) (Table 3). The colony *Cx. quinquefasciatus* were not successfully caught by HLC or tent trap when released into the insectary.

Field Tests in Senegal. In overnight tests in Senegal, the IST tent caught a range of 9.32–31.00 *An. gambiae* s.l. per trap per night across three locations (Table 4). Only one other *Anopheline* species was captured, which was a single female *Anopheles coustani.* A subsample of tent-caught *An. gambiae* s.l. from overnights were tested for species discrimination, of which 262 of 263 were identified as *An. gambiae* s.s. and one was identified as *Anopheles arabiensis.* In Kedougou overnight sampling, the tent caught 43.8 CGV mosquitoes per trap per night (Table 4). In evening crepuscular period captures in the town of Kedougou, the IST tent captured 59.4% of the mean CGV, 40% of *Ae. vittatus,* and 5% of *Ae. aegypti* relative to the HLC (Table 5).

Table 3. Number of colony-raised *Culicidae* captured in direct HLC vs. tent, 30-min trials in closed insectary

Mosquito species (release #)	Released	Exited holding container	HLC caught	Tent caught	P value
Ae. aegypti (n = 4)	160	142	97	12	0.0085
An. gambiae (n = 20)	769	701	259	152	0.0068
Cx. quinquefasciatus (n = 4)	160	104	0	3	0.391

P value based on difference in trapping efficiency via two-tailed paired t-test.

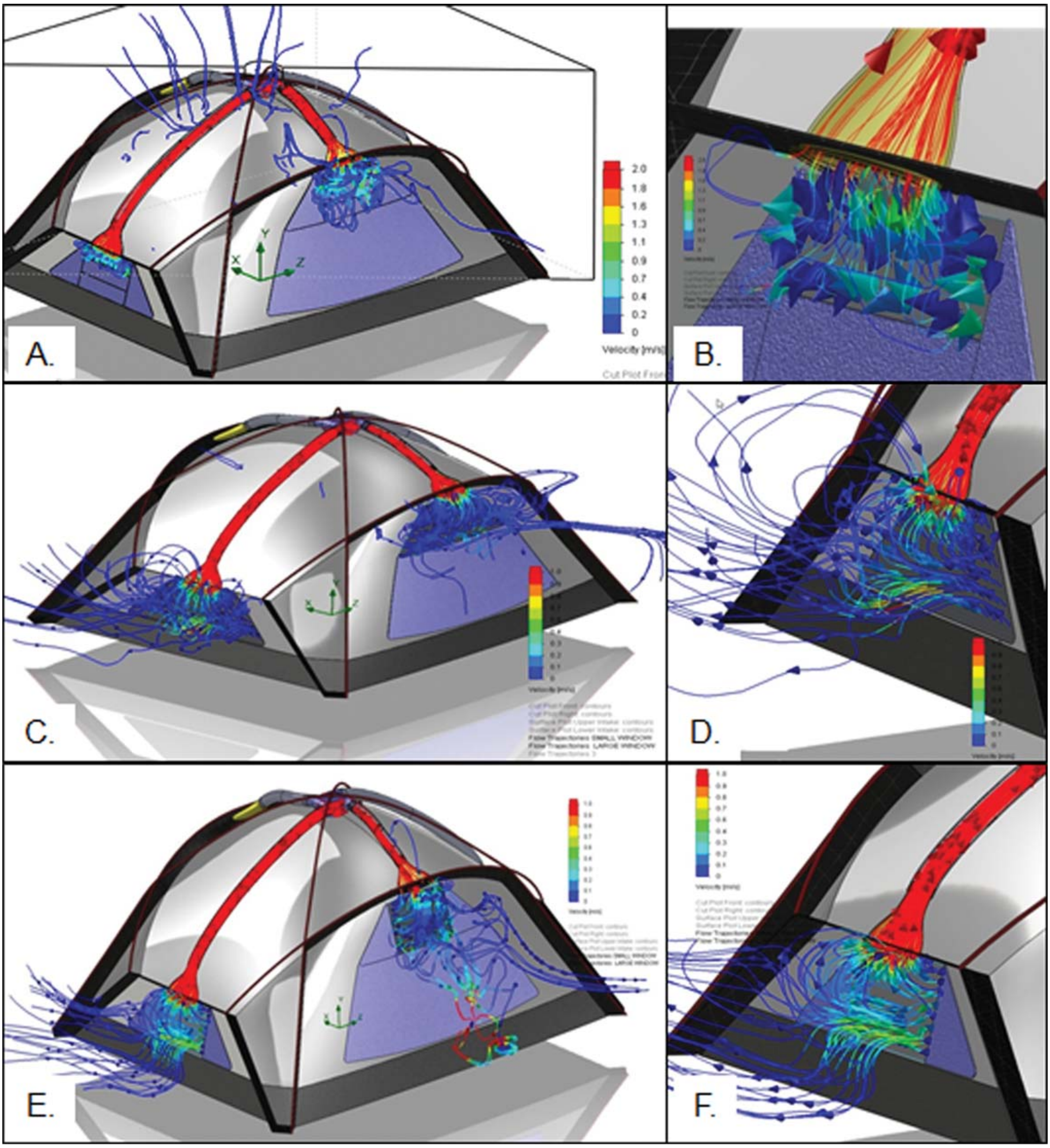

Fig. 4. Computational fluid dynamics visualization demonstrating volatile distribution and recirculation in all possible patch configurations.

This capture efficiency difference between trapping methods was only statistically different for *Ae. aegypti. Culex pipiens* complex discrimination via PCR identified 10 of 22 in a randomly selected sample as *Cx. quinquefasciatus.* The remaining were likely to be one of the other morphologically and molecularly indistinguishable CGV known to be in the area.

Discussion

Mosquitoes and other vectors host seek from a distance by using the rapidly changing CO_2 concentrations found along the edge of the odor plume (Dekker et al. 2001). Nearer the host, mosquitoes tend to follow body odor plumes, body heat, and water vapor to land on the host (Takken and Verhulst 2013). The IST trap uses these host-seeking cues by maximizing gas and volatile dispersion by a battery-powered active trapping system. This system uses a fan that creates suction at the intake ports to capture vectors following the odor plume, while simultaneously blowing air on the tent operator. This creates positive pressure inside the tent, pushing human bait-scented odor plumes that exhaust from each side of the tent.

Table 4. Number of *Anopheles gambiae* s.l. and *Culex* caught per tent per night in overnight testing in three locations in Senegal

	Anopheles gambiae s.l. – Damboucoye, Senegal	*An. gambiae* s.l. – Nathia, Senegal	*An. gambiae* s.l. – Kedougou, Senegal	*Culex* group V – Kedougou, Senegal
Trap nights	11	8	22	22
Mean (95% CI)	31.00 (16.31–45.69)	23.13 (7.75–38.50)	9.32 (6.23–12.40)	43.77 (29.87–57.67)
Max caught	81	50	25	138

Operator comfort and protection from weather and biting insects was ensured during tent use due to the utilization of modern camping tent materials. The benefit of having a durable, an insect-proof, and a weather-proof tent that was lightweight and quickly and easily deployable was apparent in our field trapping. Southeastern Senegal is a vector-rich area that historically receives an average of 1,256 mm^3 of rain in the May–October rainy season (Traore-Lamizana et al. 1996). It rained in the early evening or night approximately half of the time we performed these tests, and the rain fly prevented any rain from entering the tent. Furthermore, the base was water-resistant and prevented pooling water from soaking into the inner tent chamber. Over the duration of our sampling (1 August 2012–10 September 2012), the average ambient temperature was 29.3°C (25.7–34.6°C) and average humidity was 74.0% (55.5–85.4). The breathable tent fabric combined with the blowing fan made sleeping outdoors in the tent cooler and more comfortable than sleeping in our concrete field house. This is in contrast to a previous tent design made of plastic sheeting that was described as "very uncomfortable to sleep under" in the high ambient temperatures common in mosquito-prevalent areas (Braimah et al. 2005). The tent can be easily deployed, required less than 10 min for set up and tear down. When the vector trap is properly screwed into the manifold and the door zippers are sealed, vector exposure risk to the human operator is essentially zero. This is in comparison with HLC, whereby even after 30-min laboratory trials with the lights on, we aspirated partially blood-fed mosquitoes from the insectary at the end of several tests. It can be expected that crepuscular or all-night HLC in field conditions and stealthier wild vectors results in significant human exposure to vector bites and risk of infection with vector-borne pathogens.

The air flow simulations were performed to optimize the air intake speed and determine the amount of bait-scented air that would be recycled into the suction ports or leave the tent in odor plumes when various sections of the window mesh were covered or left open. Although the control box was designed to activate the fan at three different speeds, it was determined that the proper balance of bait-scented air recycled:exhausted was most easily adjusted by repositioning the tent fabric patch just under the suction port (Supp. Table 2 [online only]), and all tests were performed at 9V fan speed. Nevertheless, the "optimal" tent configuration between fan speed and area of exhaust mesh exposed will highly depend on ambient conditions in the field. Anecdotally, we noticed that mosquito captures were poor over evenings and nights when the weather was windy or consisted of continuous heavy rains.

In examining the comparison testing, it is of foremost importance to recognize that all sampling methods of biting vectors are biased, and only estimate the biting pressure and force of infection. The HLC is considered the gold standard, but it almost certainly overestimates true biting pressure because humans rarely sit still over hours with parts of their bodies exposed to biting vectors. The insectary data suggested that the tent may adequately capture wild host-seeking *An. gambiae,* but might poorly capture *A. aegypti* or *Cx. quinquefasciatus.* However, these experiments were performed in a closed insectary not much bigger than the tent itself, which likely interfered with odor plume formation outside the tent. Next, our colonized mosquitoes might have an altered or a diminished capacity to host seek like wild vectors. Indeed, the strain of *Cx. quinquefasciatus* we used in the laboratory tests failed to be attracted to humans either performing the HLC or operating the tent.

Crepuscular comparison sampling in Kedougou showed that, in opposition to our laboratory studies, the occupied tent does indeed attract anthropophilic CGV (including *Cx. quinquefasciatus*) mosquitoes, and all-night catches were dominated by these species (Table 4). This semiurban environment was highly conducive to the container-breeding *Culex,* with overnight catch numbers ranging from 11 to 131 per tent per night. In comparison, the villages of Damboucoye and Nathia averaged 0–1 *Culex* spp. per tent per night. While overall *Culex* numbers at these villages are likely much lower based on numbers from aspiration catches, there may be a component of density dependence affecting the catch rate of the tent, as has been

Table 5. Number of *Culicidae* from crepuscular sampling in Kedougou, Senegal, over six comparative trap sessions

	Crepuscular sampling – tent caught	Crepuscular sampling – HLC caught	*P* value
Mean *Cx.* group V caught (95% CI)	7.33 (0.57–14.09)	12.33 (7.25–17.42)	0.4004*
Mean *Ae. aegypti* caught (95% CI)	0.50 (−0.37–1.38)	9.00 (0.89–17.10)	0.0355*
Mean *Ae. vittatus* caught (95% CI)	0.40 (−0.28–1.08)	0.83 (0.040–1.62)	1

P value based on difference in trapping efficacy via Wilcoxon's two-tailed matched pairs test with Gaussian* or exact value as noted.

mentioned with the Ifakara tents (Govella et al. 2009, 2011).

Aedes activity in Kedougou followed the classic description of a strong evening crepuscular or dusk-biting period (Smith 1995, Traore-Lamizana et al. 1996). During our 2-h sampling time, most *Ae. aegypti* were caught in a 30-min time window of intense exophagic biting just as the sun was setting, and HLC was much more efficient at capturing this species. One hypothesis is that the narrow feeding interval may force the vectors into biting a more available target of the HLC operator. Alternatively, this species may resist being aspirated by the suction ports or may follow near host cues in different ways. During the HLC it was observed that *Ae. aegypti* were very small, flew in fast zigzag patterns around one's exposed leg, and were reluctant to land, in contrast to larger and directly landing *Cx. quinquefasciatus.* All-night captures in outlying villages demonstrated the tent's efficacy in capturing anthropophilic *Anopheles* species (31.0 HBR). Although we did not compare these capture rates with HLC in this study, the catch numbers/trap/night are in the same range of both indoor and outdoor HBRs reported from HLC and LTC in villages around Kedougou during the rainy season (Dia et al. 2005, Kobylinski 2011, Ndiath et al. 2011).

Interestingly, 99.8% of the identified tent captures in the villages were *An. gambiae* s.l. These data are in contrast to 94.4% *An. gambiae* s.l. that were aspirated, blood-fed and resting in houses the morning after the night catch, and suggests that some vectors are preferentially trapped by the tent, whereas others might avoid it. The second most abundant aspiration-caught vector was *An. funestus* Giles (3.0% of aspiration collections), and based on this we would have expected around 20 of this species to be captured in the tent while host-seeking during the nights before morning aspirations. However, *An. funestus* were never caught by the tent. This species is primarily described as having highly endophagic/endophilic biting/resting patterns (Russell et al. 2011). Its absence from tent collections may mean that this species avoids the tent, it host seeks differently than *An. gambiae,* or the tent is biased toward more exophagic biters (Githeko et al. 1996).

Finally, the vector trap design limited the in-trap movement and desiccation of the captured vectors, and they did not pass through the fan to enter the trap. Taxonomically relevant morphological characteristics of 98.7% ($n = 674$) of tent-caught *Anopheles* were maintained, and the majority of mosquitoes survived and were flying in the trap container when it was removed from the manifold in the morning. The high specimen quality provides the ability to use these vectors in bioassays, for colonization, or for other techniques in which live, intact mosquitoes are necessary. Although nightly captures of anthropophilic mosquitoes were in line with those reported from the same region, future direct comparative testing against HLC (both indoor and outdoor) and testing of the proportion of pathogen-infected captured vectors will be necessary to properly evaluate this new system's relative efficiency and ability to estimate pathogen transmission. However, in conclusion, the design and preliminary evaluation of the tent demonstrated how the combination of modern camping materials and CFD can be used to construct a trapping system for anthropophilic mosquitoes. The implementation, comfort, and general safety of the trapping system were significantly higher than HLC. Thus, this IST trap provides a needed entry into the realm of safe, effective mosquito trapping, and represents a significant vector surveillance breakthrough that has the potential to dramatically enhance the capability of health officials to more effectively survey, and thus control, vectors that may transmit malaria and other vector-borne diseases.

Acknowledgments

We thank the gracious and welcoming people of the villages of Damboucoye and Nathia, Senegal, without whom the frequent testing of these tents would not have been possible. We also thank Phillip Chapman for his invaluable statistical advice. We thank Greg Ebel for the *Cx. quinquefasciatus* mosquitoes and Doug Brackney for assistance in Senegal mosquito identification. Funding for this research originated from Small Business Innovation Research grant W81XWH-10-C-0214 from the U.S. Department of Defense, and research in Senegal was partly funded by grant 1R01AI094349-01A1 from the U.S. National Institutes of Health.

References Cited

Braimah, N., C. Drakeley, E. Kweka, F. Mosha, M. Helinski, H. Pates, C. Maxwell, T. Massawe, M. Kenward, and C. Curtis. 2005. Tests of bednet traps (Mbita traps) for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged insecticide treated net use. Int. J. Trop. Insect Sci. 25: 208–213.

Charlwood, J., R. Paru, H. Dagoro, and M. Lagog. 1986. Influence of moonlight and gonotrophia age on biting activity of *Anopheles farauti* (Diptera: Culicidae) from Papua New Guinea. J. Med. Entomol. 23: 132–135.

Constantini, C., G. Gibson, J. Brady, L. Merzagora, and M. Coluzzi. 1993. A new odour-baited trap to collect host-seeking mosquitoes. Parassitologia 35: 5–9.

Darbro, J., and L. Harrington. 2006. Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. J. Med. Entomol. 43: 83–92.

Dekker, T., W. Takken, and R. T. Carde. 2001. Structure of host-dour plumes influences catch of *Anopheles gambiae* ss and *Aedes aegypti* in a dual-choice olfactometer. Physiol. Entomol. 26: 124–134.

Dia, I., D. Diallo, J. B. Duchemin, Y. Ba, L. Konate, C. Costantini, and M. Diallo. 2005. Comparisons of human-landing catches and odor-baited entry traps for sampling malaria vectors in Senegal. J. Med. Entomol. 42: 104–109.

Diagne, N., D. Fontenille, L. Konate, O. Faye, M. T. Lamizana, F. Legros, J. F. Molez, and J. F. Trape. 1994. Anopheles of Senegal. An annotated and illustrated list. Bull. Soc. Pathol. Exot. 87: 267–277.

Ewert, A., and B. C. Ho. 1967. The fate of *Brugia pahangi* larvae immediately after feeding by infective vector mosquitoes. Trans. R. Soc. Trop. Med. Hyg. 61: 659–662.

Fornadel, C. M., L. C. Norris, and D. E. Norris. 2010. Centers for Disease Control light traps for monitoring *Anoph-*

eles arabiensis human biting rates in an area with low vector density and high insecticide-treated bed net use. Am. J. Trop. Med. Hyg. 83: 838–842.

Githeko, A. K., N. I. Adungo, D. M. Karanja, W. A. Hawley, J. M. Vulule, I. K. Seroney, A. V. Ofulla, F. K. Atieli, S. O. Ondijo, and I. O. Genga. 1996. Some observations on the biting behavior of *Anopheles gambiae ss, Anopheles arabiensis,* and *Anopheles funestus* and their implications for malaria control. Exp. Parasitol. 82: 306–315.

Govella, N. J., P. P. Chaki, Y. Geissbuhler, K. Kannady, F. Okumu, J. D. Charlwood, R. A. Anderson, and G. F. Killeen. 2009. A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. Malar. J. 8: 157.

Govella, N. J., P. P. Chaki, J. M. Mpangile, and G. F. Killeen. 2011. Monitoring mosquitoes in urban Dares Salaam: evaluation of resting boxes, window exit traps, CDC light traps, Ifakara tent traps and human landing catches. Parasit Vectors 4: 40.

Grieco, J. P., N. L. Achee, M. R. Sardelis, K. R. Chauhan, and D. R. Roberts. 2005. A novel high-throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. J. Am. Mosq. Control Assoc. 21: 404–411.

Ho, B. C., and A. Ewert. 1967. Experimental transmission of filarial larvae in relation to feeding behaviour of the mosquito vectors. Trans. R. Soc. Trop. Med. Hyg. 61: 663–666.

Hoel, D., D. Kline, and S. Allan. 2009. Evaluation of six mosquito traps for collection of *Aedes albopictus* and associated mosquito species in a suburban setting in North Central Florida 1. J. Am. Mosq. Control Assoc. 25: 47–57.

Kobylinski, K. 2011. Ivermectin mass drug administration to humans for malaria parasite transmission control. Ph.D. dissertation, Colorado State University, Fort Collins.

Laganier, R., F. Randimby, V. Rajaonarivelo, and V. Robert. 2003. Is the Mbita trap a reliable tool for evaluating the density of *Anopheline* vectors in the highlands of Madagascar. Malar. J. 2: 42.

Le Goff, G., P. Carnevale, E. Fondjo, and V. Robert. 1997. Comparison of three sampling methods of man-biting anophelines in order to estimate the malaria transmission in a village of south Cameroon. Parasite 4: 75–80.

Lines, J. D., C. F. Curtis, T. J. Wilkes, and K. J. Njunwa. 1991. Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. Bull. Entomol. Res. 81: 77–84.

Lutwama, J. J., J. Kayondo, H. M. Savage, T. R. Burkot, and B. R. Miller. 1999. Epidemic O'Nyong-Nyong fever in southcentral Uganda, 1996–1997: entomologic studies in Bbaale village, Rakai District. Am. J. Trop. Med. Hyg. 61: 158–162.

Lutwama, J. J., E. B. Rwaguma, P. L. Nawanga, and A. Mukuye. 2002. Isolations of Bwamba virus from south central Uganda and north eastern Tanzania. Afr. Health Sci. 2: 24–28.

Magbity, E. B., J. D. Lines, M. T. Marbiah, K. David, and E. Peterson. 2002. How reliable are light traps in estimating biting rates of adult *Anopheles gambiae* s.l. (Diptera: Culicidae) in the presence of treated bed nets? Bull. Entomol. Res. 92: 71–76.

Mathenge, E. M., G. F. Killeen, D. O. Oulo, L. W. Irungu, P. N. Ndegwa, and B. G. Knols. 2002. Development of an exposure-free bednet trap for sampling Afrotropical malaria vectors. Med. Vet. Entomol. 16: 67–74.

Mathenge, E. M., G. O. Omweri, L. W. Irungu, P. N. Ndegwa, E. Walczak, T. A. Smith, G. F. Killeen, and B. G. Knols. 2004. Comparative field evaluation of the Mbita trap, the Centers for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya. Am. J. Trop. Med. Hyg. 70: 33–37.

Mathenge, E. M., G. O. Misiani, D. O. Oulo, L. W. Irungu, P. N. Ndegwa, T. A. Smith, G. F. Killeen, and B. G. Knols. 2005. Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of *Anopheles arabiensis, An. funestus* and *Culicine* species in a rice irrigation in western Kenya. Malar. J. 4: 7.

Medica, D. L., and P. Sinnis. 2005. Quantitative dynamics of Plasmodium yoelii sporozoite transmission by infected anopheline mosquitoes. Infect Immun. 73: 4363–4369.

Monlun, E., H. Zeller, B. Le Guenno, M. Traore-Lamizana, J. Hervy, F. Adam, L. Ferrara, D. Fontenille, R. Sylla, and M. Mondo. 1993. Surveillance of the circulation of arbovirus of medical interest in the region of eastern Senegal. Bulletin de la Societe de Pathologie Exotique (1990). 86: 21.

Mutero, C., and M. Birley. 1987. Estimation of the survival rate and oviposition cycle of field populations of malaria vectors in Kenya. J. Appl. Ecol. 24: 853–863.

Ndiath, M. O., C. Mazenot, A. Gaye, L. Konate, C. Bouganali, O. Faye, C. Sokhna, and J. F. Trape. 2011. Methods to collect Anopheles mosquitoes and evaluate malaria transmission: a comparative study in two villages in Senegal. Malar. J. 10: 270.

Overgaard, H. J., S. Saebo, M. R. Reddy, V. P. Reddy, S. Abaga, A. Matias, and M. A. Slotman. 2012. Light traps fail to estimate reliable malaria mosquito biting rates on Bioko Island, Equatorial Guinea. Malar. J. 11: 56.

Pecor, J. 2012. Adult female identification key to *Culex* species of Africa (AFRICOM), with emphasis on medically important species. (http://www.wrbu.org/keys/AF_CX_A/Culex_Afrotropical_AFRICOM_A.html).

Reid, J. 1961. The attraction of mosquitoes by human or animal baits in relation to the transmission of disease. Bull. Entomol. Res. 52: 43–62.

Russell, T. L., N. J. Govella, S. Azizi, C. J. Drakeley, S. P. Kachur, and G. F. Killeen. 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J. 10: 80.

Silver, J. B. 2008. Mosquito ecology, 3rd ed. Springer, Dordrecht, The Netherlands.

Smith, T. 1995. Proportionality between light trap catches and biting densities of malaria vectors. J. Am. Mosq. Control Assoc. 11: 377.

Smith, J. L., and D. M. Fonseca. 2004. Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 70: 339.

Styer, L. M., K. A. Kent, R. G. Albright, C. J. Bennett, L. D. Kramer, and K. A. Bernard. 2007. Mosquitoes inoculate high doses of West Nile virus as they probe and feed on live hosts. PLoS Pathog. 3: e132.

Takken, W., and N. O. Verhulst. 2013. Host preferences of blood-feeding mosquitoes. Annu. Rev. Entomol. 58: 433–453.

Traore-Lamizana, M., D. Fontenille, H. G. Zeller, M. Mondo, M. Diallo, F. Adam, M. Eyraud, A. Maiga, and J.-P. Digoutte. 1996. Surveillance for yellow fever virus in eastern Senegal during 1993. J. Med. Entomol. 33: 760–765.

Wilkins, E. E., P. I. Howell, and M. Q. Benedict. 2006. IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis.* Malar. J. 5: 125.

Williams, M. C., J. P. Woodall, P. S. Corbet, and J. D. Gillett. 1965. O'nyong-Nyong fever: an epidemic virus disease in East Africa. VIII. Virus isolations from anopheles mosquitoes. Trans. R. Soc. Trop. Med. Hyg. 59: 300–306.

Wong, J., N. Bayoh, G. Olang, G. F. Killeen, M. J. Hamel, J. M. Vulule, and J. E. Gimnig. 2013. Standardizing operational vector sampling techniques for measuring malaria transmission intensity: evaluation of six mosquito collection methods in western Kenya. Malar. J. 12: 143.

World Health Organization. 2003. Malaria entomology and vector control. Learners guide. WHO, Geneva, Switzerland.

Received 10 May 2013; accepted 15 November 2013.