

Sampling Methods for Adult Malaria Vectors

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Panelists

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Moderator

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Questions & Answer

Question	Would you still recommend the inclusion of Human Landing Catches in such studies (testing the efficiency of novel traps) with its safety challenges (possibility of getting infected with malaria or other arboviruses) in the process? (to Dr Brandy)
Dr Brandy	I think I would recommend inclusion of HLC when evaluating other traps (so we have some way to compare efficacy between trap types), but after that if another method is more appropriate for a study, it is fine to use other collection methods. In some cases, it is more efficient to do so.

Question	What would panelists consider the 'perfect' trap; price, features, automated species ID, remote sensing etc.? (to all panelists)
Dr Brandy	Traps need to be cost-effective and practical as well as able to capture the mosquitoes that you are interested in. If the perfect trap is expensive and heavy, it simply will not be used and is difficult to scale up. The dream trap would capture a lot of mosquitoes, including infected mosquitoes, be cheap and straightforward to use.
Dr Amelie	It depends of the research question asked. Cows have the advantage of collecting large amount of mosquitoes, however they are not always easy to find and/or manipulate and/or bring around. The human-baited double

	net trap gives a good alternative to HLC with protection of the collector from insect bites and the advantage of being less labor-intensive as well.
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Question	There are some wing-beat frequency work that is yielding good differentiation between species, even in the species complex.
Dr Brandy	I think this type of work is really interesting. In Southeast Asia we have so many species I think this would be difficult to apply in this context. We do have some giant anophelines like An. dirus and An. hyrcanus group mosquitoes that probably have some interesting wing beats.
Dr Amelie	This is interesting to know. I guess the next step would then to be able to collect mosquitoes in large quantities, keep them alive even when collecting in remote areas and then being able to analyze each individual wing-beat frequency.

Question	What is the main vector? (to Dr Brandy)
Dr Brandy	An. dirus is the presumed major vector in this region, but there are many different species that are known to transmit - so I would say that there is likely no one main vector but many vectors acting at the same time. Malaria transmission in Southeast Asia is extremely complex, and vector diversity is part of that complexity.
Professor Theeraphap	Vector species in SE region is quite complex and absolutely need to update this aspect. For example, at least seven Anopheles species are now considered as malaria vector in Thailand.

Question	As many different species were positive for sporozoites, which species would you recommend targeting for insecticide susceptibility tests? (to Dr Brandy)
Dr Brandy	I think it is important to initially evaluate the vector composition in any area. The species that are very abundant and carrying Plasmodium should be the ones tested if you have to prioritize species. Many different species have been shown to have insecticide resistance in the GMS.
Professor Theeraphap	Even though many Anopheles species have been found resistant to various insecticides, few malaria vectors were resistant to insecticides. This is quite interesting and need more study.

Question	I do not know how it could be possible to discriminate the species before the WHO/CDC test for susceptibility. Otherwise, just specifying the complex group after molecular identification on dead and survivor mosquitoes
Dr Brandy	Yes, I think this is difficult, you could potentially separate out the knocked down/dead mosquitoes and the live mosquitoes after the test for the identification.
Professor Theeraphap	This can be done by using the isoline-F1. Once get enough mosquitoes for the test, take one or two (or even a piece of leg) for the PCR to identify the species. This is the way that we are doing at KU.

Question	You described how the traps and volunteers moved between trap sites. Did the different human volunteers also cycle between the different trapping method?
Dr Brandy	

Professor Theeraphap	To avoid the bias, the volunteer must move with the trap from one location to the other location until complete the study.
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Question	What techniques did you use to molecularly identify the Anopheles species? (To Amelie)
Dr Brandy	We used PCR to amplify and then Sanger sequenced ITS2 and CO1, which are the most widely used markers with the most reference sequences available for species identification in Anopheles.
Professor Theeraphap	We di use the multiplex PCR, ITS2 and CO1.
Dr Amelie	We used amplicon-sequencing technique based on the ITS2 and CO1 sequences

Question	An. dirus does not seem to be positive for parasites, but you have other species - large number of other species that are positive. What is the control method? (to Dr Brandy)
Dr Brandy	<p>We collected very few An. dirus (which is consistent with other groups sampling in Cambodia, An. dirus populations seem to be in decline, likely due to deforestation). I presented those tested that were collected in the cow-baited tents, but we did get (a few) P.f. positive An. dirus from some of our HLC collections in a separate study. And other people collecting in Cambodia, like Amelie, are able to get infected An. dirus mosquitoes in decent numbers.</p> <p>Since most of these species bite and rest outdoors, new methods for control are needed to address what is sometimes referred to as “residual malaria transmission”, the malaria transmission that is still occurring after other known effective methods like LLINs or IRS have been deployed.</p>

Question	Have you deployed the CBT both in village and forest? If you did, what are the differences of these two sites in collecting Anopheles? (to Dr Brandy)
Dr Brandy	Our sites were in what I would call a forest-fringe environment, but there are other studies that have done really good direct comparisons between forest and village environments in Southeast Asia and have indicated that there are many of the same species in both, but often slightly more diversity and greater densities in the forest.

Comment	I think as always the choice of sampling method depends on your purpose. Talk 3 confirms talk 1: in order to maximize sensitivity, and if you just want to sample parasites, cow trap is one of the best. But the relative sporozoite rates in different species does not tell you their relative importance as vectors. Brandy says “its the same range of species, they are all potential vectors”, but the older literature suggests that it is important to consider these categories of “primary”, “secondary” and “incidental” vector species. For this, you need to catch them seeking blood meal from humans. So I would prefer a combination of cow and double-net human bait.
Dr Brandy	I agree with Amelie’s response. I think these categorizations are only useful if they are correct, and for many of these species we don’t have enough data to determine their role in transmission (or not).
Professor Theeraphap	I agree as Amelie’s response. This study area needs to carefully update in

	order to provide a better view of the vector status.
Dr Amelie	Agreed that the choice of the sampling method depends on the research question. However, we should be careful in using the primary, secondary and incidental categories since they introduce biases and people often do not even screen other species anymore despite the fact that large differences can exist between sites, mosquito populations, and seasons as well as due to ecological and epidemiological changes over time.

Comment	Would you recommend human double net trap over human decoy trap based on your experience with both traps? (to Professor Theeraphap)
Professor Theeraphap	Double net trap is quite simple and easy to set up whereas human decoy trap needs more time and quite complicate to prepare (boil the water, set up the tent, set up the ventilation, and others). Also we will get the unbroken and nice specimens from the double net trap whereas human decoy trap needs some times to remove specimens. One major advantage from the human decoy trap is that there is no or small risk for the volunteer in getting malaria

Comment	Thank, Dr. Brandy. If the mosquitoes's gut is not collected, the mosquitoes that is positive with occyest is missed? may be if you screen the mosquitoes's gut, your positive number will be more. (to Dr Brandy)
Dr Brandy	We took the head and thorax because we were interested in potentially infectious mosquitoes, but you are correct that we might miss mosquitoes with only oocyst stage parasites in the midgut.

Question	Hi Brandy: Great study! My interpretation: The advantage of the cow trap is that it catches large numbers. Probably it maximises the number of infected femakes, if you just want to sample parasite DNA. But it does not answer Prof Indra's question! We have known for many years that in this region there are many incidental vectors: species that occasionally bite humans and occasionally get infected, but are incapable of maintaining local trasnmission on their own. Not sure whether your data challenge this. That's why I ask whether you looked for parasites in the abdomen as well as head and thorax. (to Dr Brandy)
Dr Brandy	We need both lab infection studies to determine the vector competence of different species as well as screening many species in the field for infection (regardless of whether we believe them to be vectors or not). There are many areas where the presumed major vectors haven't been collected in years but transmission is occurring, that indicates that other species are sustaining transmission. If there are a bunch of not so great vectors that are sometimes biting and getting infected but are at very high densities and biting when people are active and outdoors, they can sustain transmission.
Dr Amelie	By looking at the parasite in the head and thorax we can say that the parasites developed to the sporozoite stage and thus also went through the oocyst stage. Looking at the abdomen only is more tendentious as a positive PCR could indicate parasite present in the blood meal only.

Question	How do you take care of cows post trials/studies as there is chance of diseases transmitted to Cows? (to Drs Brandy and Amelie)
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Dr Brandy	In our case, the cows were not protected from bites, but were essentially standing in a tent in the same locations they would be standing and exposed to the same bites. I don't think our collections in any way increased their chance of acquiring any disease. We were also always working with the owner of the cow (who is often a collector too), and they look after the cows during and after the experiments. If animals were displaying any sign of distress, they were switched out.
Dr Amelie	The cows are rented and thus taken care of by their owner post-trial. In addition, the inner net protect the cow from insect bites, same as for the human volunteer in the human baited trap net. We used the same double net trap set-up (expect for the size) to be able to calculate an anthropophily index not biased by using two different collection method.

Question	Can u explain more about evenness (to Dr Brandy)
Dr Brandy	Species evenness is a measure of how evenly distributed individuals are among species categories. Low evenness indicates that only a few species are very abundant while the other species present are in low numbers. High evenness indicates that the different species that are present occur in similar numbers/abundance. This measure is hard to get to without collecting many mosquitoes in as unbiased a way as possible.

Question	Did you process just the head and thorax, or the whole female?
Dr Brandy	The head and thorax only
Dr Amelie	The head and thorax only

Question	Thanks Brandy- great talk. Is there a concern that the mosquitoes you are catching using CBT are more likely to be zoophilic, and may not bite humans? (Though clearly some do as they are infected) (to Dr Brandy)
Dr Brandy	We know that these mosquitoes are biting humans because of the Plasmodium infection, and we have also done some blood-typing and found mixed animal-human blood meals. I would be concerned if we had not collected positive mosquitoes. Also, many of these mosquitoes are generalist feeders (will bite whatever they have access to) rather than having extremely strong preference one way or the other. I think that host preference is not as fixed a trait as we often state it to be. Both Amelie's and my data from different studies indicate that most of the species seeking bloodmeals from humans and cattle were the same.

Question	Pk positive in anopheles is seen in which type of community? (to Dr Brandy)
Dr Brandy	It was in Pursat province in a house on the edge of a large swath of forest.

Question	Did you capture non-Anopheles mosquitos? (Aedes for example?) - With the cow trap?
Dr Brandy	Aedes not as commonly, but we do collect plenty of Culex mosquitoes. We were not interested in these so we did not keep them.
Professor Theeraphap	
Dr Amelie	We captured non-anopheles mosquitoes with the cow trap. When we captured the 3,920 anopheles females, we had an additional 13,980 non-

	anopheles mosquitoes from which 1,368 were Aedes mosquitoes.
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Question	We have hilly areas in Bangladesh where we couldn't be able to hlc, so sir can we use others two that you have shown now? (to Prof. Theeraphap)
Professor Theeraphap	Of course, the two new traps are great in the area where human landing catch is not possible. As I suggested, these two new techniques need more works in comparison with the human landing catch before make any final conclusion.

Question	Dr. Theeraphap, thank you for the wonderful talk. Do you mean that higher rainfall is associated to higher anopheles abundance or only restricted to some species? (to Professor Theeraphap)
Professor Theeraphap	Yes, especially with An. dirus complex. This species is more abundance during the rainy season due to the huge availability of breeding habitats (quiet water e.g. animal footprint or small containers that hold rain water in the forest or forest fringe area) which are totally different from other malaria vectors. We have collected hundreds of An. dirus and An. maculatus during the rainy season.

Question	Did you map the Zoophilic and Anthropophilic tendency of the Anopheles species in your trap catch studies? (to Dr Brandy)
Dr Brandy	Again, I think most of these species are generalist feeding, maybe slightly on the zoophilic side. Bloodmeal analysis has indicated a lot of multiple host feeding behavior and we can see that many of the same species seek blood meals from humans and other hosts like cattle.

Question	Which species was the positive individual?
Dr Brandy	From 4 different studies we did in Cambodia, 22 of 37 species that we collected were positive for P.f. (admittedly often at very low rates). Some that were frequently infected were An. barbirostris, An. maculatus, and An. hyrcanus s.l..

Question	Why are there no seasonal changes in mosquito diversity?
Dr Brandy	There were some seasonal changes, but there was consistently high diversity in each site throughout the year

Question	Did the same volunteers iterate between the different mosquito capture systems? (to Professor Theeraphap)
Professor Theeraphap	See above

Question	Was difference in findings in trap efficiency between two countries due to a difference in the relative abundance of different An. species between the two countries?
Professor Theeraphap	Not sure the question? If you look at the number of Anopheles mosquitoes collected from Vietnam, this number was so low, poor sampling size. The trap comparison analysis from Vietnam may not be appropriated.

Question	To all speakers - were Pf, Pv and Pk the only Plasmodia you detected?
Dr Brandy	We picked up a few monkey malarias. But P.f., P.f., and P.k. were the only ones we detected. I know that in some assays (which are usually

	developed to detect P.f. and P.v only), P.o. and P.m. are not as easily amplified so it possible that we missed them.
Professor Theeraphap	
Dr Amelie	Po samples were detected as well. Other Plasmodia have been detected too but need further analyses.

Question	I am also collecting anopheles from South eastern area of bangladesh, there is presence of malaria cases but getting very large amount of anopheles in the animal house other than human house? (to Dr Amelie)
Dr Amelie	You could have zoophilic malaria vectors too, checking the presence of malaria parasite in the heads and thoraces would give some indication.

Question	Will the human decoy trap also attract other biting insects, such as horseflies? The presence of other biting insects inside the trap usually pose a problem for collectors. (to Professor Theeraphap)
Dr Brandy	
Professor Theeraphap	We found very few other insects trapped by human decoy trap, mostly mosquitoes. Remember! Horseflies are diurnal insect and we did the study during the night time.

Question	How do you collect the mosquitoes from the sticky paper in the HDT? Can you check them for Plasmodium detection? (to Professor Theeraphap)
Professor Theeraphap	<p>Good question, but no, we did not do the parasite detection.</p> <p>To remove the specimens, please follow;</p> <ol style="list-style-type: none"> 1. cover the sticky surface of the trap with “food wrap”. (This must be transparent. 2. Use sharp scissors or a scalpel to carefully cut the sheet of sticky plastic and food wrap free from the trap. 3. The surface of the trap should still feel warm. If possible, check this with an infra-red spot thermometer; it should be at least 30°C. 4. Place white paper on a table and examine the sheet on this to highlight any mosquito specimens sandwiched between the sticky plastic and the food wrap. 5. Cut out each mosquito sample using scissors. Double check the sheet to make sure all mosquito specimens have been located and removed. 6. Use a microscope to identify species, flipping the “sandwich” to examine both sides of the specimen. 7. If necessary, specimens can be removed using glue solvent (vegetable oil may also be suitable for removing samples). Place the plastic “sandwich” in enough solvent to cover the sample. Leave for a minute and gently move around the sample in the solvent using forceps. Hold the sample in position with one pair of forceps, then gently pull at the top layer of plastic with another pair of forceps until it is loose. Peel away gently, allowing the solvent to flood over the mosquito specimen. Leave for a minute longer and use soft forceps to very gently lift the mosquito off the sticky plastic. Place the specimen on tissue or cotton and allow the solvent to evaporate, then store in tube with silica gel for subsequent molecular work. Alternatively, keep the solution wet in 70% ethanol for further examination under the microscope. (SOP-APMEN)

Question	How many vector which engender to be malaria?
Dr Brandy	Many different species. We need more data from many screened mosquitoes from more locations to really answer that question.
Professor Theeraphap	In Thailand, at least seven Anopheles are regarded as malaria vectors (molecular identification)
Dr Amelie	In the collection presented, we found 14 different mosquito species (molecular identification) with human malaria parasites.

APMEN would like to thank all of our panelists for their time and sharing their knowledge.

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