USAID | *StopPalu+*

ANNUAL REPORT OF ENTOMOLOGICAL SURVEILLANCE ACTIVITIES (NOVEMBER 2017–OCTOBER 2018)

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Abbreviations

CDC	Centers for Disease Control and Prevention
CREC	Entomology Research Center of Cotonou
CSP	Circumsporozoite Protein
ELISA	Enzyme Immunosorbent Assay
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GOG	Government of Guinea
HLC	Human Landing Collection
IRS	Indoor Residual Spraying
Kdr	Knockdown Resistance (mutation)
LLIN	Long-lasting Insecticide-treated Net
MICS Palu	Multiple Indicator Cluster Survey (on Malaria)
MOH	Ministry of Health
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
UGANC	Gamal Abdel Nasser University of Conakry
USAID	United States Agency for International Development
WHO	World Health Organization

Summary

The U.S. President's Malaria Initiative (PMI) Program Component (*StopPalu+*) activities are implemented to reduce the malaria burden in Guinea.[1] These activities include building in-country capacity to carry out entomological surveys. During November 1, 2017–October 31, 2018 (the period covered by this annual report), *StopPalu+* supported the National Malaria Control Program's (NMCP's) field activities and supported the operation of the insectary and laboratory for conducting entomological investigations. The focus of *StopPalu+*'s entomological activities is to continue to support the NMCP in developing a national vector control strategy to (1) control the growth of vectors across the country, (2) identify areas affected by insecticide resistance, and (3) implement the most effective interventions.

During this second year of supporting entomological activities in Guinea, *StopPalu+* accomplished the following:

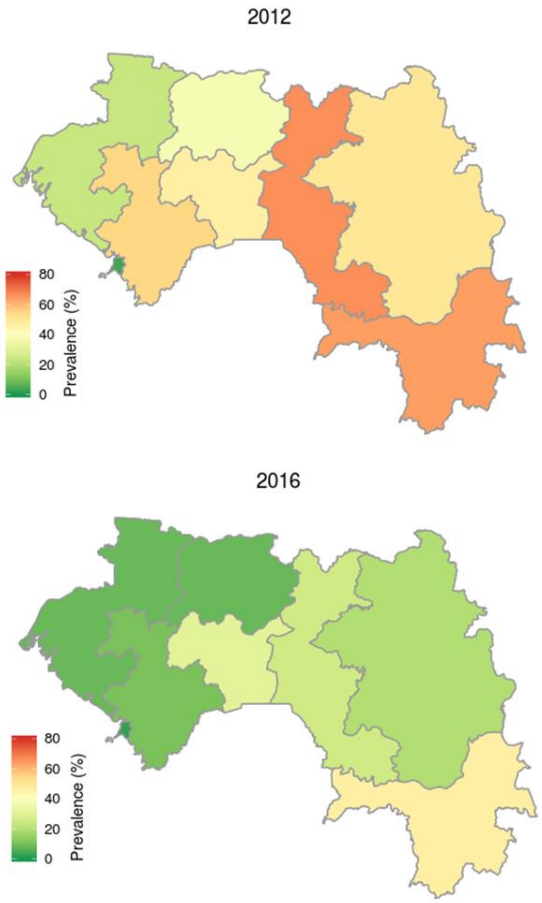
- Continued to support the laboratory and the insectary
- Continued to support the care for two pairs of rabbits housed at the Gamal Abdel Nasser University of Conakry to support the production of mosquitoes
- Continued to support the breeding of Kisumu strain mosquitoes for multiple generations
- Continued to visit the sentinel sites in Boké, Kankan, Kissidougou, Labé, Dabola, and Faranah where mosquitoes were collected to facilitate national entomological surveillance
- Conducted monthly study of vector seasonality in Boké and Faranah

[1] *StopPalu+* is the five-year follow-on project to the original *StopPalu* project. *StopPalu+* started in December 2017 and will run through December 2022.

1 Context

Over the past five years, Guinea has made enormous progress in malaria control, leading to a reduction in malaria prevalence in children under five years of age, (***Figure 1***), annual malaria incidence, and in-patient deaths[2,3,4]. Progress was such that in 2016, the Government of Guinea (GOG) received an Award of Excellence from the Alliance of African Leaders Against Malaria for the country's efforts against malaria.[5] Much of this progress is due to the GOG's leadership and commitment to scale up key interventions against malaria, backed by substantial external financial support, specifically the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) and the U.S. President's Malaria Initiative (PMI).

Figure 1: The prevalence of malaria in children under 5 years



Guinea's malaria efforts face several challenges, however, including major transmission heterogeneity between regions and districts (*préfectures*); a constrained malaria commodity procurement supply chain; uneven technical and program planning, implementation, and management capacity at national, regional, and district levels; and mounting insecticide resistance.[6] These challenges invariably impact programmatic intervention coverage and effectiveness. Consequently, malaria remains the most burdensome communicable disease in Guinea: the entire 12.1 million population is at risk, with approximately 1 million cases reported in 2016, accounting for 31% of outpatient visits.[3]

The main malaria vector in Guinea is *Anopheles gambiae* sensu lato (s.l.), specifically *An. gambiae* sensu stricto (s.s.), *An. coluzzii*, *An. arabiensis*, and *An. melas*. The *An. funestus* complex is also present, but studies published to date indicate that *An. funestus* is less prevalent than the *An. gambiae* complex [7,8]. Vector control interventions implemented in Guinea include the mass distribution of long-lasting insecticide-treated mosquito nets (LLINs) and, to a very limited extent, indoor residual spraying (IRS) with propoxur, deltamethrin, and pirimiphos-methyl.

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[2] National Institute of Statistics and MEASURE DHS (2012) *Demographic and Health Survey (DHS) and Multiple Indicator Cluster Survey (MICS) 2012*. Conakry, Guinea, pp. 1–528.

[3] National Institute of Statistics (2016) *Multiple Indicator Cluster Survey with Malaria Component (MICS-Palu) 2016*. Conakry, Guinea.

[4] Ministry of Health (MOH) (2017) *National Strategic Plan for Malaria Control 2018–2022*. Conakry, Guinea, p. 1–69.

[5] https://au.int/en/pressreleases/19675/african-heads-state-celebrate-progress-against-malaria-african-leaders-malaria

[6] MOH (2017) *Performance Review of the Strategic Plan for Malaria Control 2013–2017*. Conakry, Guinea, p. 1–75.

[7] Vezenegho, S. B., B. D. Brooke, R. H. Hunt, M. Coetzee, and L. L. Koekemoer. "Malaria vector composition and insecticide susceptibility status in Guinea Conakry, West Africa." Medical and veterinary entomology 23, no. 4 (2009): 326-334.

[8] Carnevale, Pierre, J. C. Toto, P. Guibert, M. Keita, and Sylvie Manguin. "Entomological survey and report of a knockdown resistance mutation in the malaria vector Anopheles gambiae from the Republic of Guinea." Transactions of the Royal Society of Tropical Medicine and Hygiene 104, no. 7 (2010): 484-489.

The resistance status of malaria vectors in Guinea was recently evaluated in six sites (Boké, Labé, Maferinyah, Faranah, Kankan, and Kissidougou).[9] Resistance to DDT, permethrin, alpha-cypermethrin, and lambda-cyhalothrin was detected in all *An. gambiae* s.l. populations tested; populations were susceptible to deltamethrin and bendiocarb in two of the three sites sampled. The *Kdr* (knockdown resistance) West mutation was widespread, and frequency was 60% or more in all sites sampled; the *Ace-1* mutation was present in low levels. There is limited information on the insecticide resistance status in *An. funestus*, with complete susceptibility to deltamethrin and malathion (unspecified dose) reported.[10]

2 *StopPalu*+ Results Framework

PMI's main operational platform for malaria efforts from 2013 onward has been the USAID Guinea *StopPalu* project (2013–2017) and the current follow-on project *StopPalu*+ (December 2017–December 2022), both led by RTI International. *StopPalu*+'s goal is to assist the GOG to achieve the PMI target of reducing malaria-related morbidity and mortality by 75% compared with 2016 levels. This will be achieved through (1) increasing the use of LLINs by the population; (2) increasing the use of intermittent preventive treatment of malaria in pregnancy during antenatal care visits; (3) increasing prompt care-seeking and treatment; (4) increasing the number of full doses of seasonal malaria chemoprevention that are delivered in a timely manner; (5) increasing community involvement in and support for malaria prevention, care, and treatment activities; and (6) strengthening the technical capacity of the National Malaria Control Program (NMCP) to manage, implement, and monitor prevention, care, and treatment activities.

The overarching aims of entomological activities supported by *StopPalu*+ are to strengthen the country's entomological capacity and generate malaria vector data that will help the NMCP and stakeholders to develop and implement Guinea's vector control strategy.

Entomological activities supported by *StopPalu*+ have three main objectives during this second year:

Objective 1: Strengthen national entomological capacity by:

- Supporting the operation of an entomology laboratory and insectary at the Gamal Abdel Nasser University of Conakry (UGANC)
- Maintaining a colony of *An. gambiae* (Kisumu strain)
- Train UGANC students and the NMCP interns in maintenance techniques for the insectary
- Supporting the Vector Control Technical Working Group meetings

Objective 2: Conduct entomological surveillance by:

- Collecting mosquitoes in selected sentinel sites
- Assessing vector distribution and abundance
- Assessing vector seasonality
- Assessing vector biting behavior
- Species identification and sporozoite index

Objective 3: Determine insecticide resistance of malaria vectors by:

- Carrying out susceptibility testing in sentinel sites

[9] Keita, K. et al. (2017) Species identification and resistance status of *Anopheles gambiae* s.l. (Diptera: Culicidae) mosquitoes in Guinea. *Journal of Medical Entomology, 54*, 677–681.
[10] AngloGold Ashanti (2007) Unpublished.

- Carrying out intensity tests for deltamethrin, permethrin, and alpha-cypermethrin at different doses
- Collecting semi-gravid mosquitoes for chromosomal analysis

3 Achievements

3.1 Objective 1: Strengthen national entomological capacity

Since the official opening of the entomology laboratory and insectary at UGANC, *StopPalu+* has conducted several activities, including (1) supporting the operation of the laboratory and insectary, (2) maintaining a susceptible *An. gambiae* s.s. population (Kisumu strain); (3) holding meetings, organized by the staff, with the NMCP Vector Control Unit; and (4) training student interns.

3.1.1 Supporting the functioning of the entomology laboratory and insectary at UGANC

The laboratory and insectary were established on the UGANC grounds in November 2016 with support from Professor Martin Akogbeto (Entomology Research Center of Cotonou, Benin [CREC]); both have been fully functional ever since. Laboratory and insectary setup have been fully described in *StopPalu*'s Year 5 (fiscal year [FY] 2017) Entomological Report. The activities carried out during FY 2018 are as follows:

Laboratory

In 2016, UGANC provided physical space to *StopPalu* to establish an entomology laboratory and insectary. *StopPalu*, with PMI support, supported the rehabilitation and conversion of these buildings. The laboratory consists of two large air-conditioned and ventilated rooms equipped with shelves and large tables. The equipment and materials available are used to conduct entomological surveillance and insecticide resistance tests. The first room serves as the entomological laboratory and the second room is devoted to molecular biology and biochemistry testing.

Figure 2: UGANC entomology laboratory and insectary

Insectary

The insectary, managed by *StopPalu+*, is a large room divided into two compartments (compartments A and B). Compartment A is assigned as the adult breeding room. It includes two large shelves that hold approximately 20 adult holding cages. Compartment B of the insectary is temperature-controlled and assigned for the breeding of mosquito larvae. The first breeding activities started with breeding the *An. gambiae* s.s. Kisumu strain of mosquitoes brought from Cotonou (Benin). An animal enclosure is annexed to the insectary and houses two pairs of rabbits used to feed adult mosquitoes.

The entomological equipment and laboratory materials are summarized in the tables in the Annex.

3.1.2 Maintaining the An. gambiae (Kisumu strain) colony

An. gambiae s.s. are being reared according to standard procedures[11] (***Figure 3***):

Figure 3: Breeding *An. gambiae s.s.* at the UGANC insectary

- Female mosquitoes are allowed to take a blood meal from a rabbit immobilized on a device designed for this purpose and placed at the top of the cage in which the mosquitoes are housed. Mosquitoes are fed almost seven days after emergence.
- Mosquitoes are transferred into an oviposition tub after the second feeding; egg laying happens after 24 or 48 hours.
- Eggs are transferred into labeled freshwater tanks filled with 500 ml of spring water to allow hatching.
- Hatched larvae are transferred to tanks and classified according to their stage of development; tetramin fish food is fed to mosquito larvae.
- When mosquitoes reach the nymphal stage, they are transferred to cages for emergence.
- Adults are kept in cages until they are needed for study and they are fed with a solution of water and sugar ad libitum.

During FY 2018, the insectary produced 13 generations of mosquitoes.

3.1.3 Training student interns

Figure 4: Trainees at the insectary

Similar to FY 2017, UGANC students interested in a career in entomology and the NMCP interns were trained in all mosquito breeding techniques and insectary maintenance; in FY 2018 11 interns were selected and completed the internship.

3.1.4 Support for the Vector Control Technical Working Group meetings

During FY 2018, *StopPalu*+ supported the NMCP's Vector Control Unit in organizing eight monthly meetings. During these meetings, vector control activities for the month/quarter were reviewed and discussed, and activities for the months/quarters ahead were planned.

[11] Das, S., Garver, L., and Dimopoulos, G (2007) Protocol for the breeding of mosquitoes (*A. gambiae*). *Journal of Visualized Experiments*, 221.

3.2 Objective 2: Conduct entomological surveillance

3.2.1 Collecting mosquitoes in selected sentinel sites

Entomological surveillance activities were conducted to investigate the distribution, abundance, seasonality, behavior, rate of infection, and the status of insecticide resistance in malaria vectors in eight districts: Boké, Faranah, Labé, Lola, Kankan, Kissidougou, Maferinyah, and Siguiri. These districts were selected to represent the four main malaria endemicity zones in Guinea. In each district, three villages with the highest malaria prevalence were selected as sentinel sites (***Table 1***).

During the reporting period (November 2017–October 2018), *StopPalu+* and the NMCP team conducted visits to priority sites (Labé, Kankan, Kissidougou) and in secondary sites (Siguiri, Lola, Maferinyah), once each. In addition, the team conducted a study of vector seasonality in Boké and Faranah.

During the visits to the 24 villages, three mosquito collection methods were used: (1) human landing catch (HLC), (2) Centers for Disease Control and Prevention (CDC) light traps, and (3) pyrethrum spray catch (PSC) (***Tables 2*** and ***3***). These methods were used to gather information on the vector species composition, abundance (for mapping), physiological status, and host-seeking behavior.

Table 1: Sentinel sites for entomology surveillance activities

Designation	Administrative Regions	District	Villages (Sentinel sites)
Priority sites	Moyenne Guinée	Labé	Banty, Thialy, Toutouroun
	Haute Guinée	Kankan	Dalabani, Balandou, Makonon
	Guinée Forestière	Kissidougou	Gbangbadou, Kérédou, Tongbèkoro
Secondary sites	Haute Guinée	Siguiri	Dankakoro, Tiguigbiri, Tabakoro
	Guinée Forestière	Lola	Gama konikoni, Togbanata, Weyekore
	Basse Guinée	Maferinyah	Madinagbe, Fandie, Moribaya
Sites to study seasonality	Basse Guinée	Boké	Kaboye, Dioumaya, Guilere
	Haute Guinée	Faranah	Balayani, Foulaya, Tindo

Table 2: Summary of the frequency of collections and number of houses sampled in both seasonal sites for FY2018†

Sites	Location	Schedule of visits					
		Visit #1 (February to June)			Visit #2 (July to October)		
		HLC	LT	PYR	HLC	LT	PYR
	Labé						
	Banty,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Thialy,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Toutouroun	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Kankan						
	Dalabani,	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
	Balandou	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
	Makonon	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
	Kissidougou						
	Gbangbadou,	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
	Kérédou,	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
	Tongbèkoro	2 (1)	2 (1)	1 (10)	1 (2)	2 (1)	1 (10)
Subtotal (priority)		18	18	90	18	18	90
	Lola						
	Dankakoro,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Tiguigbiri,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Tabakoro	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Maferinyah						
	Gama konikoni	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Togbanata,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Weyekore	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Siguiri						
	Madinagbe	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
	Fandie,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)

	Moribaya	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
Subtotal (secondary)		18	18	90	18	38	90
Total		36	36	180	36	36	90
One house per village were sampled over two nights for HLCs and Light traps, while ten houses were sampled for PSCs per village over one day for a total of 10 different houses per month; HLC = human landing collection; LT = CDC light trap; PYR = pyrethrum spray collection.							

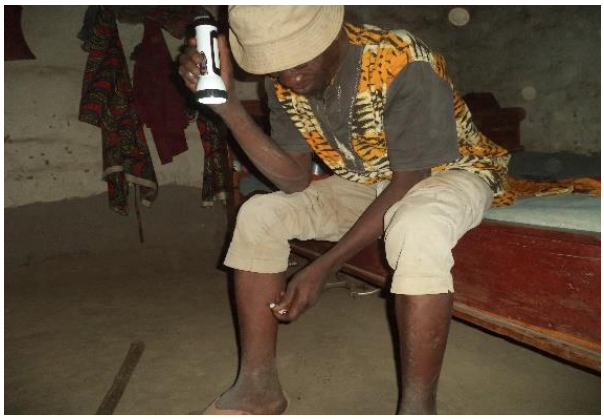
Table 3: Summary of the frequency of collections and number of houses sampled in both seasonal sites in FY2018†

	Schedule of visits																	
	February			May			June			July			August			October		
Location	**HLC**	**LT**	**PYR**	**HLC**	**LT**	**PYR**	**HLC**	**LT**	**PYR**	**HLC**	**LT**	**PYR**	**HLC**	**LT**	**PYR**	**HLC**	**LT**	**PYR**
Boké																		
Kaboye,	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	*	*	*	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
Dioumaya	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	*	*	*	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
Guilere	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	*	*	*	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)
Faranah																		
Balayani	2 (2)	2 (2)	1 (10)	2 (2)	2 (2)	1 (10)	2 (2)	2 (2)	1 (10)	2 (2)	2 (2)	1 (10)	2 (2)	2 (2)	1 (10)	*	*	*
Foulaya	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	*	*	*
Tindo	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	2 (1)	2 (1)	1 (10)	*	*	*
Total number of houses sampled	12	12	60	12	12	60	12	12	60	6	6	30	12	12	60	6	6	30
† One house per village were sampled over two nights for HLCs and Light traps, while ten houses were sampled for PSCs per village over one day for a total of 10 different houses per month; HLC = human landing collection; LT = CDC light trap; PYR = pyrethrum spray collection; * No collection occurred at this time																		

3.2.2 Assessing vector distribution and abundance

Human landing catch (HLC)

HLCs are a standard for determining human-vector contact and for specifically assessing mosquito biting behavior. HLC was carried out in one house per village for two nights per visit. The activity was carried out inside and outside houses in each of the 24 selected villages. Two collectors were situated inside and outside of a house in each village from 1800 to 0700 hrs; a first pair of collectors worked from 1800 to 0100 hrs and a second pair of collectors worked from 0100 to 0700 hrs. At each hour collectors switched from indoors to outdoors and outdoor to indoors. Collectors used individual tubes to capture mosquitoes that were trying to bite their exposed legs; the tubes were packaged in different plastic bags for each collection hour to determine the timing of aggressive behavior throughout the night. The collected mosquitoes were returned to the laboratory for species identification by standard morphological keys.



HLC outside a house

HLC inside a house

Of 10,301 mosquitoes collected across the sites, 8,636 (84%) were *Anopheles*, including 8,401 *An. gambiae* and 235 *An. funestus*. Among *Anopheles* mosquitoes collected, 4,593 (53.2%) (range across sites: 21.6–68.6%) were caught indoors.

Among priority sites, the Kankan sites collected most *Anopheles* mosquitoes—620 (55.0%) indoors and 507 (45.0%) outdoors. Among the secondary sites, most Anopheles mosquitoes were captured in Maferinyah—1,633 (55.7%) indoors and 1,299 (44.3%) outdoors. While in 14 sites more *An. gambiae* s.l. were caught indoors, in 2 sites (Guilere, Tiguigbiri) more were collected outdoors than indoors; in 8 sites, comparative numbers of *An. gambiae* s.l. were caught indoors and outdoors. The overall mean of mosquitoes collected through HLC results are presented in **Table 4.**

Table 4: Mean abundance and distribution of mosquitoes collected indoors and outdoors using HLC over 4 nights (2 nights over 2 visits) of collection in one house per village.

Districts	Sentinel sites	*An. gambiae s.l.*		*An. funestus s.l.*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
Kankan	Dalabani	64.00	58.25	0.75	0.50	0.75	0.75	0.25	0.75
	Makono	55.25	41.00	0.00	0.25	19.00	9.50	0.00	2.50
	Balandou	35.00	26.75	0.00	0.00	13.50	1.75	1.00	2.00
	Mean	**51.42**	**42.00**	**0.25**	**0.25**	**11.08**	**4.00**	**0.42**	**1.75**
Kissidougou	Gbangbadou	24.00	18.25	0.00	0.00	0.50	0.25	0.00	0.00
	Kérédou	6.00	5.75	0.00	0.00	1.00	0.25	0.00	0.00
	Tongbèkoro	40.25	29.75	0.00	0.00	0.50	0.50	1.25	0.75
	Mean	**23.42**	**17.92**	**0.00**	**0.00**	**0.67**	**0.33**	**0.42**	**0.25**
Labé	Banty	16.25	8.75	0.00	0.00	2.75	5.25	0.00	2.75
	Tountouroun	7.25	5.75	0.00	0.00	1.50	4.75	1.50	2.75
	Thialy	22.75	23.25	0.00	0.00	0.75	0.00	0.00	0.00
	Mean	**15.42**	**12.58**	**0.00**	**0.00**	**1.67**	**3.33**	**0.50**	**1.83**
Lola	Gama koni	20.25	9.25	0.00	0.00	2.25	0.25	0.00	0.00
	Tokpanata	51.75	53.50	0.00	0.00	0.25	1.00	0.25	0.00
	Weyakore	19.25	12.00	0.00	0.00	2.00	0.50	0.00	0.00
	Mean	**30.42**	**24.92**	**0.00**	**0.00**	**1.50**	**0.58**	**0.08**	**0.00**
Maferinyah	Madinagbe	163.50	110.75	0.00	0.00	0.00	0.00	0.00	0.00
	Fandie	96.75	79.00	0.00	0.00	0.00	0.00	0.00	0.00
	Moribaya	148.00	135.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	**136.08**	**108.25**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Boké	Kaboye	6.25	5.25	0.00	17.50	17.50	17.00	0.00	0.00
	Guilere	6.50	8.50	0.00	0.00	23.25	25.50	0.00	0.00
	Dioumaya	7.50	7.00	0.00	0.00	15.75	24.00	0.00	0.00
	Mean	**6.75**	**6.92**	**0.00**	**5.83**	**18.83**	**22.17**	**0.00**	**0.00**
Faranah	Balayani	115.00	119.00	5.75	10.50	0.75	1.00	0.75	7.50
	Foulaya	128.25	130.00	7.75	15.50	1.25	1.25	0.50	0.50
	Tindo	78.50	78.25	9.00	8.25	1.75	2.75	1.25	1.75
	Mean	**107.25**	**109.08**	**7.50**	**11.42**	**1.25**	**1.67**	**0.83**	**3.25**
Siguiri	Dankakoro	9.50	5.75	0.50	0.00	37.50	43.75	0.00	0.00
	Tiguigbiri	1.75	3.75	0.00	0.00	31.75	60.00	0.00	0.00
	Tabakoro	1.00	1.25	0.00	0.00	0.75	0.50	0.00	0.00
	Mean	**4.08**	**3.58**	**0.17**	**0.00**	**23.33**	**34.75**	**0.00**	**0.00**
Overall mean (indoor and outdoor)		**52.96**	**45.95**	**1.11**	**2.50**	**5.00**	**4.58**	**0.32**	**1.01**
Percentage		**54%**	**46%**	**31%**	**69%**	**52%**	**48%**	**24%**	**76%**
Overall species mean		**49.46**		**1.80**		**4.79**		**0.67**	

CDC light traps

CDC light traps were used to collect indoor and outdoor host-seeking mosquitoes. In each village, two houses were randomly selected and sampled for one night, with one trap located indoors (near sleeping spaces, if possible) and a second trap located outdoors from 1800 to 0700 hrs.

Of 2,431 mosquitoes collected across sites, 1,093 (45%) were *Anopheles* mosquitoes—1,078 *An. gambiae* s.l. and 15 *An. funestus* s.l. Among collected Anopheles mosquitoes, 635 (58.1%) (range across sites: 9.5–100%) were caught indoors. Of all the *An. gambiae* collected using CDC light traps indoors and outdoors, 37.2% and 23.1%, respectively, were caught in Kankan. The overall mean of mosquitoes collected by CDC light trap collection are presented in **Table 5**.

A CDC light trap placed outside to capture mosquitoes

A CDC light trap placed inside to capture mosquitoes

Table 5: Mean abundance and distribution of mosquitoes collected indoors and outdoors using Light traps over 4 nights (2 nights over 2 visits) of collection in one house per village.

Districts	Sentinel sites	CDC light trap results FY 2018							
		An. gambiae		*An. funestus*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
Kankan	Dalabani	11.5	6.0	0.0	0.0	11.5	16.5	0.5	1.5
	Makono	40.5	41.5	0.0	0.0	14.5	2.5	3.5	1.5
	Balandou	64.0	5.0	0.0	0.0	22.0	13.0	0.5	0.5
	Mean	**38.7**	**17.5**	**0.0**	**0.0**	**16.0**	**10.7**	**1.5**	**1.2**
Kissidougou	Gbangbadou	1.0	0.5	0.0	0.0	4.0	3.0	0.0	0.0
	Kérédou	15.5	11.0	0.0	0.0	12.5	10.5	2.5	1.0
	Tongbèkoro	15.0	10.5	0.0	0.0	4.0	3.5	0.5	0.5
	Mean	**10.5**	**7.3**	**0.0**	**0.0**	**6.8**	**5.7**	**1.0**	**0.5**
Labé	Banty	0.0	0.0	0.0	0.0	12.5	2.5	0.0	5.5
	Tountouroun	0.0	0.0	0.0	0.0	13.5	32.5	1.0	7.5
	Thialy	7.0	0.5	0.0	0.0	0.5	2.0	0.0	0.0
	Mean	**2.3**	**0.2**	**0.0**	**0.0**	**8.8**	**12.3**	**0.3**	**4.3**
Lola	Gama Koni	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
	Tokpanata	14.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weyakore	3.5	21.5	0.0	0.0	1.0	1.0	0.0	0.0
	Mean	**6.2**	**7.2**	**0.0**	**0.0**	**0.7**	**0.3**	**0.0**	**0.0**
Maferinyah	Madinagbe	3.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
	Fandie	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0

Districts	Sentinel sites	CDC light trap results FY 2018							
		An. gambiae		*An. funestus*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
	Moribaya	4.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**2.7**	**2.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Boké	Kaboye	9.5	14.5	0.0	0.0	18.5	24.5	0.0	0.0
	Guilere	10.0	10.5	0.0	0.0	20.5	28.0	0.0	0.5
	Dioumaya	8.0	11.0	0.0	0.0	18.5	25.0	0.0	1.0
	Mean	**9.2**	**12.0**	**0.0**	**0.0**	**19.2**	**25.8**	**0.0**	**0.5**
Faranah	Balayani	33.5	21.0	3.0	0.5	6.0	8.0	1.5	6.5
	Foulaya	48.5	22.5	2.5	1.5	8.5	10.5	5.0	8.5
	Tindo	10.5	4.5	0.0	0.0	8.5	8.5	0.0	4.0
	Mean	**30.8**	**16.0**	**1.8**	**0.7**	**7.7**	**9.0**	**2.2**	**6.3**
Siguiri	Dankakoro	4.5	9.5	0.0	0.0	21.0	27.0	0.0	0.0
	Tiguigbiri	3.0	28.5	0.0	0.0	64.5	67.0	0.0	0.0
	Tabakoro	3.5	2.5	0.0	0.0	11.5	10.5	0.0	0.0
	Mean	**3.7**	**13.5**	**0.0**	**0.0**	**32.3**	**34.8**	**0.0**	**0.0**
Overall mean (indoor / outdoor)		**13.0**	**9.5**	**0.2**	**0.1**	**11.4**	**12.3**	**0.6**	**1.6**
Percentages		**58%**	**42%**	**73%**	**27%**	**48%**	**52%**	**28%**	**72%**
Overall species mean		**11.2**		**0.2**		**11.9**		**1.1**	

Pyrethrum spray catches

Pyrethrum spray catches consists of collecting adult mosquitoes inside the houses to determine species present indoors and their physiological state. Collections were conducted in the 24 selected villages. Depending on the size of the village, 10 houses/huts were selected choosing an arbitrary start location and skipping one or two houses and using the second or third house. In each house, the floor was covered with white sheets to collect knocked-down mosquitoes, with collection teams receiving permission from all households before spraying. The collections took place from 0700 to 1000 hrs. An operator sprayed the indoor area with a pyrethroid insecticide after taking all necessary precautions, including removing all food and other sensitive materials. Houses were sprayed from the outside inward through the openings to prevent mosquitoes from escaping. After spraying, all windows and doors were kept closed for 15 minutes. After 15 minutes, the sheets were taken out of the house to collect mosquitoes that had fallen on the sheets. Mosquitoes were brought to the laboratory where their species were identified, and their physiological status (unfed, fed, gravid, and semi-gravid) was determined.

Pyrethrum collection in Kankan and Boké

Of the 1,664 female Anopheles mosquitoes collected across sites, all were *An. gambiae* s.l. Of the 1,664 female *An. gambiae* s.l., 1,013 (60.9%) were fed, 162 (9.7%) were unfed, 489 (29.4%) were semi-gravid and none were gravid. The results of the pyrethrum spray catch and resident reports of LLIN possession are presented in **Table 6.**

Table 6: Mean abundance and distribution of mosquitoes collected using the pyrethrum spray catches (indoors) over 2 nights (1 night over 2 visits) of collection in ten houses per village.

Districts	Pyrethrum spray catch results FY 2018											
	Sentinel sites	*An. gambiae s.l.*				*An. funestus s.l.*				*Resident reports of LLIN possession*		
		unfed	fed	SG	G	unfed	fed	SG	g	Beds	LLIN	Residents
Kankan	Dalabani	0.0	7.1	0.3	0.0	0.0	0.0	0.0	0.0	0.5	0.4	0.9
	Makono	0.0	4.5	0.1	0.0	0.0	0.0	0.0	0.0	0.5	0.4	1.0
	Balandou	0.0	5.0	0.3	0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.1
	Mean	**0.0**	**5.5**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.4**	**1.0**
Kissidougou	Gbangbadou	0.4	0.5	1.4	0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.6
	Kérédou	0.2	1.1	1.6	0.0	0.0	0.0	0.0	0.0	0.7	0.3	2.3
	Tongbèkoro	0.1	2.3	2.3	0.0	0.0	0.0	0.0	0.0	0.6	0.5	2.1
	Mean	**0.2**	**1.3**	**1.8**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.6**	**0.4**	**2.0**
Labé	Banty	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	1.0	1.0	2.0
	Tountouroun	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.8
	Thialy	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.9	2.1
	Mean	**0.0**	**0.3**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**	**1.0**	**2.0**
Lola	Gama Koni	0.2	1.4	1.6	0.0	0.0	0.0	0.0	0.0	0.8	0.5	1.8
	Tokpanata	0.2	0.9	1.2	0.0	0.0	0.0	0.0	0.0	0.6	0.5	1.3
	Weyakore	1.9	1.9	2.4	0.0	0.0	0.0	0.0	0.0	0.8	0.6	1.6
	Mean	**0.8**	**1.4**	**1.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**	**0.5**	**1.6**
Maferinyah	Madinagbe	0.0	2.5	2.6	0.0	0.0	0.0	0.0	0.0	1.5	1.1	2.5
	Fandie	0.0	2.0	2.4	0.0	0.0	0.0	0.0	0.0	0.8	0.4	1.6
	Moribaya	0.0	3.1	2.8	0.0	0.0	0.0	0.0	0.0	0.6	0.4	1.3
	Mean	**0.0**	**2.5**	**2.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**	**0.6**	**1.8**
Siguiri	Dankakoro	0.4	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.4	1.9
	Tiguigbiri	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.3

Districts	Sentinel sites	Pyrethrum spray catch results FY 2018										
		An. gambiae s.l.				*An. funestus s.l.*				*Resident reports of LLIN possession*		
		unfed	**fed**	**SG**	**G**	**unfed**	**fed**	**SG**	**g**	**Beds**	**LLIN**	**Residents**
	Tabakoro	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.1	1.1
	Mean	**0.2**	**0.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.3**	**1.4**
Seasonality sites												
Boké	Kaboye	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	2.5	1.2	6.1
	Guilere	0.0	0.5	0.2	0.0	0.0	0.0	0.0	0.0	2.5	2.4	5.8
	Dioumaya	0.2	0.4	0.3	0.0	0.0	0.0	0.0	0.0	2.5	1.1	5.5
	Mean	**0.1**	**0.4**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**2.5**	**1.6**	**5.8**
Faranah	Balayani	2.1	8.1	2.3	0.0	0.0	0.0	0.0	0.0	3.0	2.0	6.3
	Foulaya	1.9	5.4	1.6	0.0	0.0	0.0	0.0	0.0	3.2	2.7	6.9
	Tindo	0.6	2.0	1.0	0.0	0.0	0.0	0.0	0.0	2.8	1.2	4.7
	Mean	**1.5**	**5.2**	**1.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**3.0**	**2.0**	**6.0**
Overall mean		0.4	2.1	1.0	0.0	0.0	0.0	0.0	0.0	1.2	0.9	2.7

Larvae collection

As part of mosquito surveillance efforts, a larval collection was performed in each sentinel village. The goal of this collection was to gather information on mosquito breeding sites and collect mosquitoes for insecticide susceptibility tests. Collections were performed during the rainy season (May–July 2018), with sites selected during searches at the sentinel sites, either during visits or when driving to and from the sentinel sites. Pupae and larvae were collected from different water bodies using pans and dippers, and they were then raised in the laboratories located in the Regional and Prefectural Health Directorates and health centers. The larvae were fed with ground fish food and transferred to standard insect rearing cages after pupation; they received a 5%–10% glucose solution ad libitum before being tested.

Collection of larvae in the sentinel sites

3.2.3 Assessing vector seasonality

The sites Boke and Faranah were sampled to understand the vector seasonality.

The results are presented in the table below:

Table 7: Seasonal sum of *An. gambiae s.l.* and *An. funestus s.l.* collected in Boké and Faranah villages as determined using HLCs

	Sentinel Site	February				May				June				July				August				October			
		An. gambiae s.l.		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	0	0	0	0	0	0	0	0	6	5	0	0	*	*	*	*	11	11	0	0	8	5	0	0
	Guilere	1	0	0	0	0	0	0	0	3	7	0	0	*	*	*	*	10	13	0	0	12	14	0	0
	Dioumaya	0	0	0	0	0	0	0	0	2	5	0	0	*	*	*	*	13	13	0	0	15	10	0	0
	Total	1	0	0	0	0	0	0	0	11	17	0	0	*	*	*	*	34	37	0	0	35	29	0	0
Faranah	Balayani	52	110	5	12	83	80	1	4	155	193	5	18	79	41	6	4	91	52	6	4	*	*	*	*
	Foulaya	46	86	6	37	128	106	3	1	170	219	7	16	79	47	7	6	90	62	8	2	*	*	*	*
	Tindo	57	77	22	19	65	38	3	0	98	142	7	6	45	28	0	3	49	28	4	5	*	*	*	*
	Total	155	273	33	68	276	224	7	5	423	554	19	40	203	116	13	13	230	142	18	11	*	*	*	*
*** No collection occurred at this time**																									

Table 8: Seasonal sum of *An. gambiae s.l.* and *An. funestus s.l.* collected in Boké and Faranah as determined using CDC light traps

	Sentinel Site	February				May				June				July				August				October			
		An. gambiae s.l.		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*		*An. gambiae s.l.*		*An. funestus s.l.*	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	0	0	0	0	0	0	0	0	4	3	0	0	*	*	*	*	8	11	0	0	7	5	0	0
	Guilere	0	0	0	0	0	0	0	0	3	4	0	0	*	*	*	*	8	10	0	0	8	8	0	0
	Dioumaya	0	0	0	0	0	0	0	0	1	3	0	0	*	*	*	*	10	15	0	0	5	4	0	0
	Total	0	0	0	0	0	0	0	0	8	10	0	0	*	*	*	*	26	36	0	0	20	17	0	0
Faranah	Balayani	2	4	4	0	0	2	0	0	48	27	2	1	8	4	0	0	9	5	2	0	*	*	*	*
	Foulaya	13	7	2	2	2	1	0	0	60	27	3	1	10	3	0	0	12	5	0	0	*	*	*	*
	Tindo	0	0	0	0	0	2	0	0	9	3	0	0	5	2	0	0	7	2	0	0	*	*	*	*
	Total	15	11	6	2	2	5	0	0	117	57	5	2	23	9	0	0	28	12	2	0	*	*	*	*
*** No collection occurred at this time**																									

Table 9: Seasonal sum of An. gambiae s.l. and An. funestus s.l. collected in Boké and Faranah as determined using pyrethrum spray catches

	Sentinel Site	**February**				**May**				**June**				**July**				**August**				**October**			
		An. gambiae				*An. gambiae*				*An. gambiae*				*An. gambiae*				*An. gambiae*				*An. gambiae*			
		Unfed	Fed	SG	G	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	0	0	0	0	0	0	0	0	0	0	0	0	*	*	*	*	0	2	0	0	0	2	0	0
	Guilere	0	0	0	0	0	0	0	0	0	1	0	0	*	*	*	*	0	3	0	0	0	5	0	0
	Dioumaya	0	0	0	0	0	0	0	0	1	1	0	0	*	*	*	*	2	3	0	0	0	3	0	0
	Total	0	0	0	0	0	0	0	0	1	2	0	0	*	*	*	*	2	8	0	0	0	10	0	0
Faranah	Balayani	7	13	0	0	7	23	0	0	21	79	0	0	1	26	0	0	5	20	0	0	*	*	*	*
	Foulaya	5	17	0	0	6	28	0	0	10	34	0	0	5	15	0	0	12	13	0	0	*	*	*	*
	Tindo	0	5	0	0	0	10	0	0	1	12	0	0	0	10	0	0	10	2	0	0	*	*	*	*
	Total	12	35	0	0	13	61	0	0	32	125	0	0	5	41	0	0	27	35	0	0	*	*	*	*
*** No collection occurred at this time**																									

3.2.4 Assessing vector biting behavior

Analysis of the results of the vectors' biting behavior using HLC, recorded during hour-long intervals, shows biting behavior inside and outside houses in the sites between 1900 and 0600 hrs. Whereas peak indoor biting was observed between 0200 and 0300 hrs, peak outdoor biting was observed between 0000 and 0300 hrs (***Table 7, Figure 5***). Note, peak biting times indoors and the outdoors varied substantially between sites (***Figure 6***).

Figure 5: Total Biting behavior *An. gambiae s.l.* (as determined by HLC)

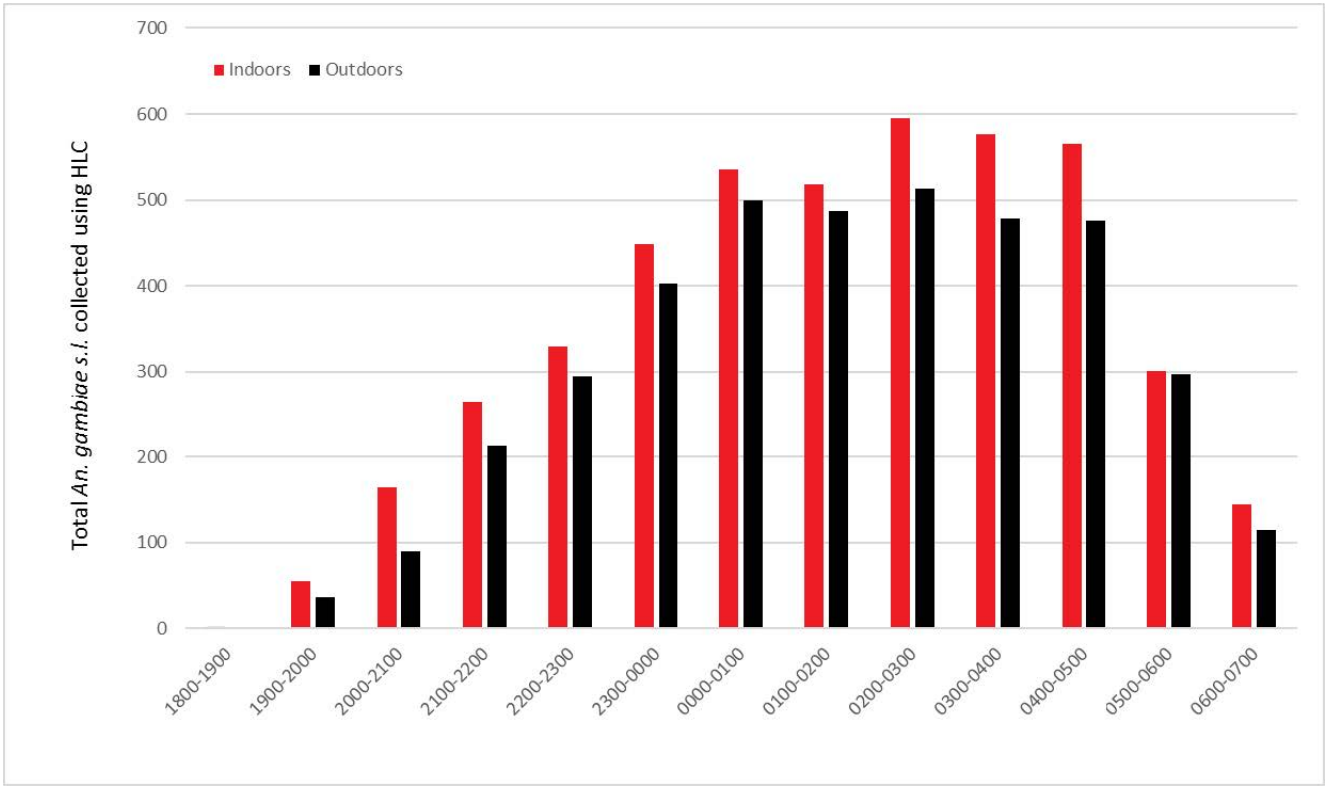
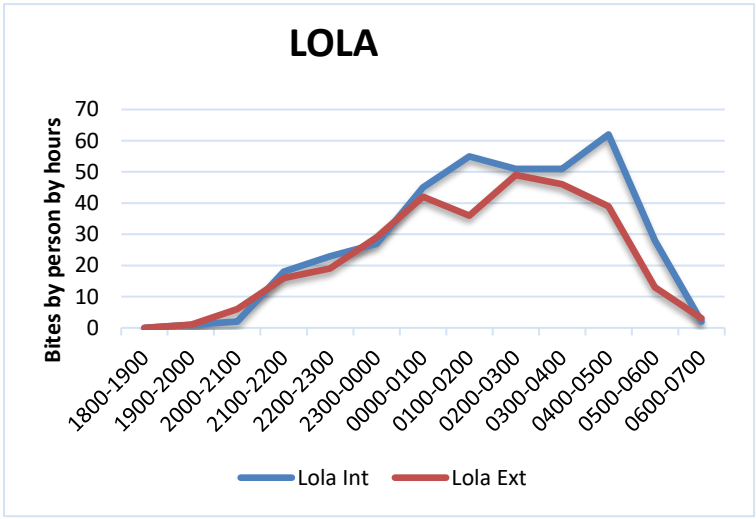
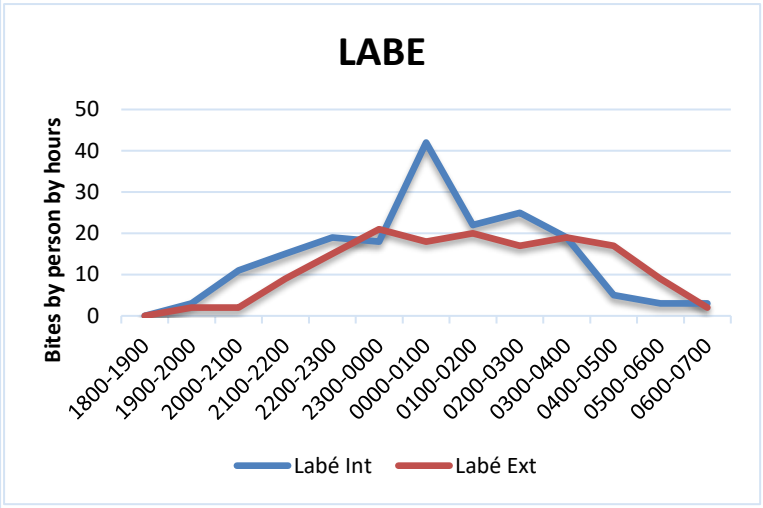


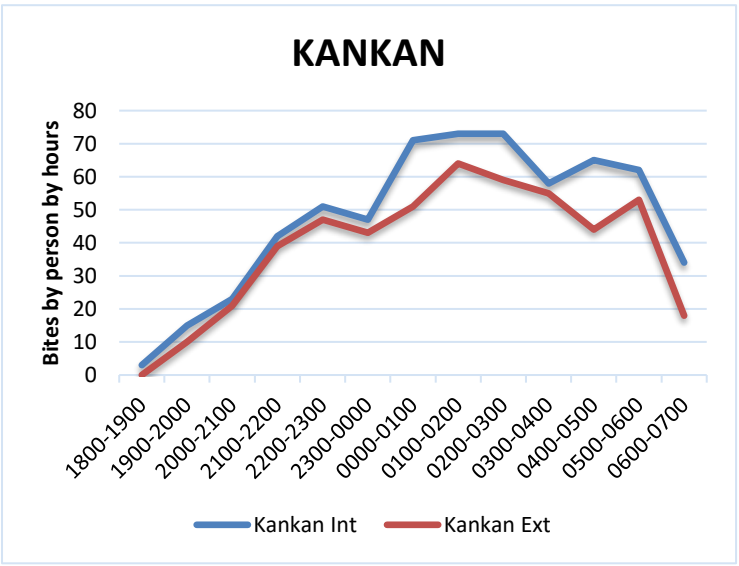
Table 10: Total *An. gambiae* s.l. biting behavior as determined using HLCs

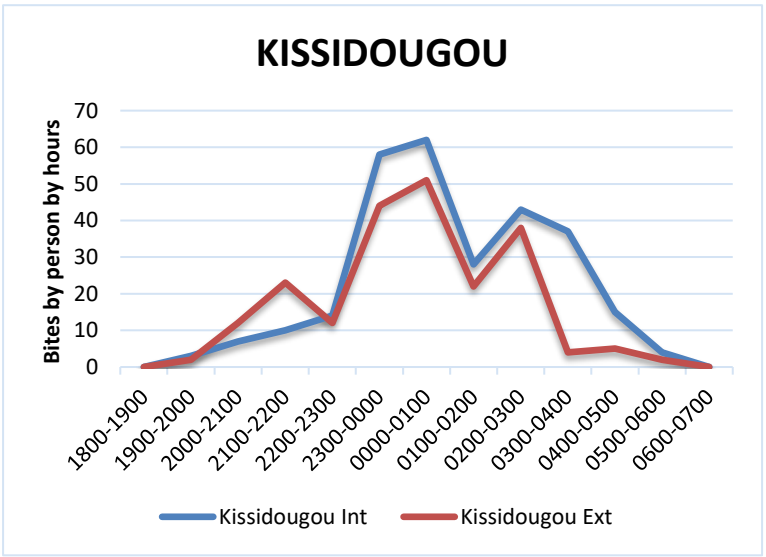
Time intervals	Lola		Labé		Kankan		Kissidougou		Maferinyah		Siguiri		Boké		Faranah		Percentage	
	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
1800-1900	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	100	0
1900-2000	1	1	3	2	15	10	3	2	24	1	0	0	0	0	9	20	60	40
2000-2100	2	6	11	2	23	21	7	12	76	5	2	3	4	2	39	39	65	35
2100-2200	18	16	15	9	42	39	10	23	114	40	4	3	5	3	55	80	55	45
2200-2300	23	19	19	15	51	47	14	12	122	92	5	4	5	7	91	99	53	47
2300-0000	27	29	18	21	47	43	58	44	152	148	11	7	9	5	127	106	53	47
0000-0100	45	42	42	18	71	51	62	51	153	151	10	8	12	9	140	170	52	48
0100-0200	55	36	22	20	73	64	28	22	156	154	11	9	10	10	163	172	52	48
0200-0300	51	49	25	17	73	59	43	38	212	156	4	3	14	13	173	178	54	46
0300-0400	51	46	19	19	58	55	37	4	232	194	2	3	11	14	166	143	55	45
0400-0500	62	39	5	17	65	44	15	5	224	202	0	2	6	7	188	160	54	46
0500-0600	28	13	3	9	62	53	4	2	88	84	0	1	3	9	113	126	50	50
0600-0700	2	3	3	2	34	18	0	0	80	72	0	0	2	4	23	16	56	44
Total	**365**	**299**	**185**	**151**	**617**	**504**	**281**	**215**	**1,633**	**1,299**	**49**	**43**	**81**	**83**	**1,287**	**1,309**	**58.3%**	**41.6%**

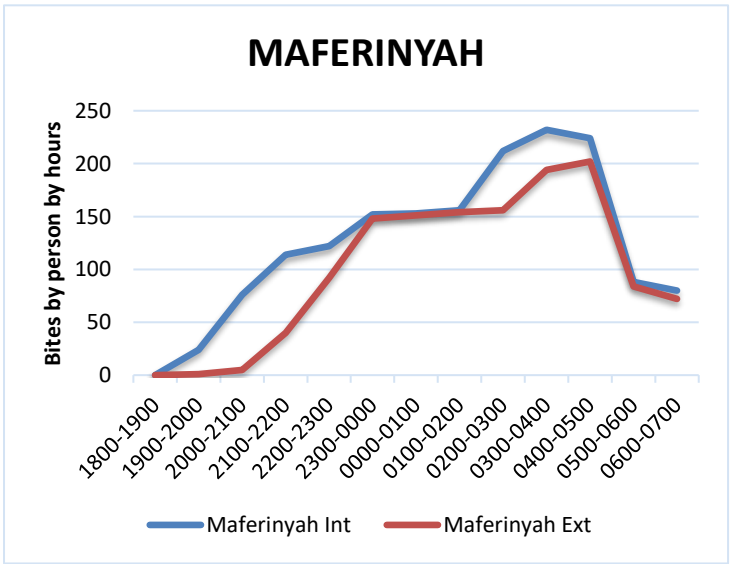
Figure 6: Biting behavior *An. gambiae* s.l. (as determined by HLC, across districts)

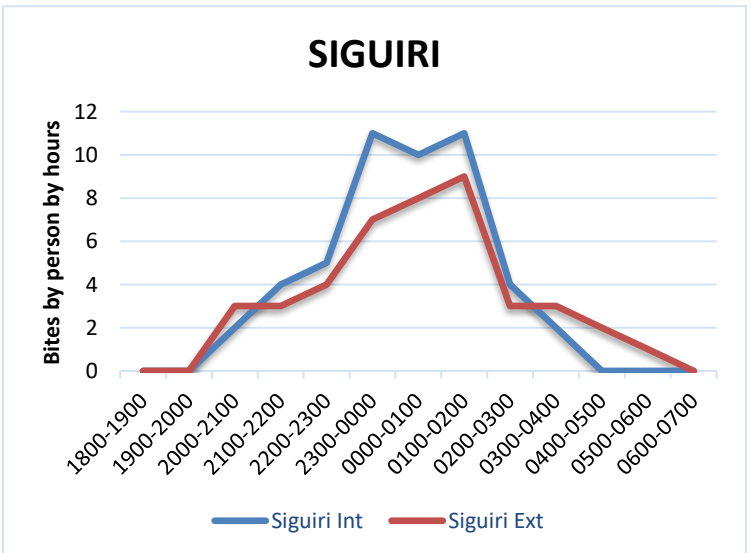


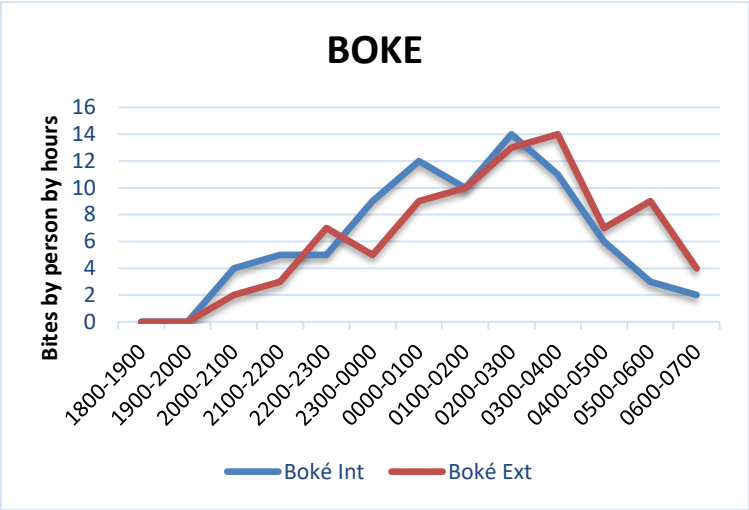


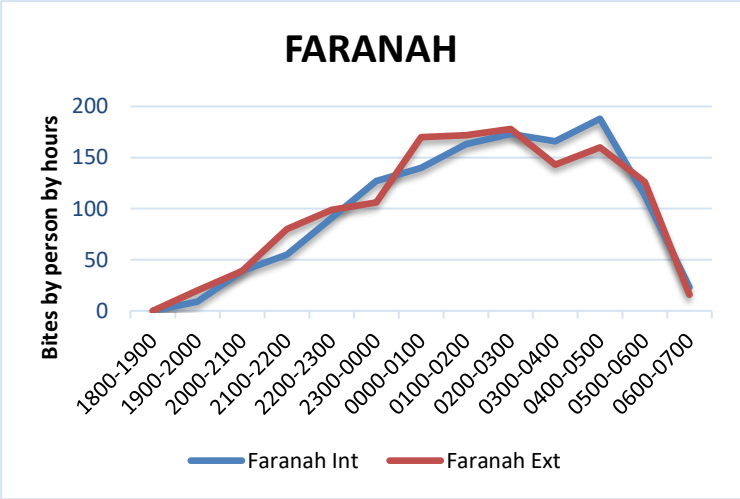












Anopheles identification, coding, and packaging on site in Faranah

3.2.5 Species identification and sporozoite index

All *Anopheles* mosquitoes collected by the different sampling methods (HLCs, CDC light traps, and pyrethrum spray catches) in the 24 villages were morphologically identified by genus and species using the standard keys. *Anopheles* were placed individually in tubes containing silica gel, numbered, coded, and preserved in refrigerators at UGANC.

PCR species identification

The molecular identification of species of the *An. gambiae* complex in Guinea was made using polymerase chain reaction (PCR) *SINE200* according to the protocol of Santolamazza et al (2008).[12]

A total of 1,410 *An. gambiae* s.l. females from eight villages in Guinea were analyzed using PCR *SINE*. The results of molecular tests performed are presented in ***Table 11***

- Three species were found in the sites: *An. gambiae* sensu strictu (s.s.), *An. coluzzii*, and *An. arabiensis.*
- In seven locations out of the eight (7/8), *An. gambiae* was the major species, with frequencies of 97.46% (Dabola), 84.35% (Faranah 2), 59.71% (Kankan), 98.48% (Kissidougou), 99.5% (Labé), 98.02% (Faranah 1), and 72.73% (Lola).
- In Siguiri, *An. coluzzii* was the predominant species, with a frequency of 55.05%.

[12] Santolamazza, F. Mancini, E., Simard, F. Qi, Y., Tu, Z., and della Torre, A (2008) Insertion polymorphisms of *SINE200* retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria Journal, 7*, 163.

- *An. arabiensis* was recorded with low frequencies only in the areas of Dabola (1.52%), Kissidougou (1.02%), and Labé (0.51%).
- Hybrids of *An. gambiae* and *An. coluzzii* were noted in Dabola (0.51%) and Siguiri (2.02%).

Table 11: Distribution of *An. gambiae* s.l. species from districts in Guinea

Sites	N tested	*An. gambiae s.l.*			
		An. gambiae s.s.	*An. coluzzii*	*Hybrid – An. gambiae s.s. / An. coluzzii*	*An. arabiensis*
Dabola	197	192	1	1	3
Faranah 2	115	97	18	0	0
Kankan	206	123	83	0	0
Kissidougou	197	194	1	0	2
Labé	198	197	0	0	1
Siguiri	198	85	109	4	0
Faranah 1	101	99	2	0	0
Lola	198	144	54	0	0
Total	**1,410**	**1,131**	**268**	**5**	**6**

ELISA to determine the sporozoite index

As recommended by the work plan, 400 *An. gambiae* s.l. from the seasonality sites (Boké and Faranah) were tested with enzyme-linked immunosorbent assay-circumsporozoite protein of *Plasmodium falciparum* (ELISA-CSP), of which six were positive, for an average sporozoite index of 1.5% (***Table 12)***. The positive sporozoite of Boké was collected by HLC and the among the five positives of Faranah, 2 were from HLC and three from PYR.

Table 12: *An. gambiae* s.l. sporozoite index

Sites	Species	Capture method		Number tested	Number positive	Positive (%)
		HLC	PYR			
Boké	*An. gambiae* s.l.	50	0	50	1	2.0
Faranah	*An. gambiae* s.l.	300	50	350	5	1.4
Total		**350**	**50**	**400**	**6**	**1.5**

3.3 Objective 3: Determine insecticide resistance of malaria vectors

3.3.1 Monitoring phenotypic resistance

Resistance tests were carried out on nulliparous female mosquitoes derived from larvae and pupae collected at the district sites as per standard World Health Organization (WHO) protocol, i.e., 1-hour exposure of mosquitoes to insecticide-treated filter papers in WHO test tubes and monitored for mortality 24 hours after exposure.[13] Six insecticides were tested: deltamethrin 0.05%, alpha-cypermethrin, and permethrin 0.75% for sensitivity/susceptibility; and pirimiphos-methyl 0.25%, propoxur 0.1%, and bendiocarb 0.1%. For each test, 125 mosquitoes were used: 100 mosquitoes to test insecticide efficacy; 25 mosquitoes as

[13] WHO (2016) *Test procedures for monitoring insecticide resistance in malaria mosquitoes.* WHO, Geneva, Switzerland.

control. The control group mosquitoes were not exposed to any insecticide but monitored for 24 hours. The susceptibility and intensity tests were performed once for all sites

WHO test tube bioassay showed that sampled vector populations are resistant to permethrin and alpha-cypermethrin, and susceptible to deltamethrin; also, the tests showed susceptibility to deltamethrin, pirimiphos-methyl, bendiocarb, and propoxur (***Table 13)***.

Table 13: Summary of insecticide susceptibility testing of mosquito vectors (% mortality; 100 mosquitoes were used to test the insecticide and 25 as control group)

Insecticides	Concentration	Boke		Labe		Kankan		Kissidougou		Lola		Faranah		Siguiri		Maferinyah	
		T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
Deltamethrin	0.05%	**100**	0	**98**	0	**100**	0	**100**	0	**100**	0	**100**	0	**100**	**0**	100	**4**
Permethrin	0.75%	**94**	0	**97**	0	**95**	0	**91**	0	**72**	0	**96**	0	**91**	**0**	94	**0**
Alpha-Cyprethrin	0.05%	**88**	0	**75**	0	**90**	0	**95**	0	**92**	0	**94**	0	**100**	**0**	90	**0**
Pirimiphos-methyl	0.25%											**100**	0				
Bendiocarb	0.1%											**100**	4				
Propoxur	0.1%											**100**	0	100			
T = mortality in mosquitoes exposed to insecticide; C = mortality in control mosquitoes																	

Susceptibility (98 – 100% mortality); Possibility of resistance (90-97%); Resistance (<90%)

Intensity tests in CDC bottles

To measure the intensity of mosquito resistance to insecticides that are advocated for use in LLINs, intensity tests were conducted with deltamethrin, permethrin, and alpha-cypermethrin. For this test, 250 ml bottles are coated with the insecticides, dried, and then mosquitoes are exposed for 30 minutes (diagnosis time) to determine susceptibility. Various doses are tested: the diagnostic dose is used to define the susceptibility/resistance, and vector survival at doses that are more concentrated (2×, 5×, 10×), and the diagnostic dose indicates resistance. In the control groups, the mosquitoes were not exposed to any insecticide. ***Table 14*** shows a summary of test results to date.

Table 14: Summary of insecticide intensity tests with CDC bottle bioassay (% mortality). 100 mosquitoes were used to test the insecticide and 25 as control group

Insecticides	Doses	Boke		Labe		Kankan		Kissdougou		Maferinyah	
		T	C	T	C	T	C	T	C	T	C
Deltamethrin	1×	100	4	98	0	98	0	100	0	100	4
	2×	100	0	100	0	100	0	100	0	100	0
	5×	100	0	100	0	100	0	100	0	100	0
	10×	100	0	100	0	100	0	100	4	100	0
Permethrin	1×	94	0	97	0	95	0	91	0	94	0
	2×	100	0	100	0	100	0	100	0	100	0
	5×	100	0	100	0	100	0	100	0	100	0
	10×	100	0	100	0	100	0	100	0	100	4
Alpha-cypermethrin	1×	88	0	75	0	95	0	95	0	90	0
	2×	98	0	100	0	100	0	100	0	100	0
	5×	100	0	100	0	100	0	100	0	100	0
	10×	100	0	100	0	100	0	100	0	100	0
T = mortality in mosquitoes exposed to insecticide; C = mortality in control mosquitoes											

Susceptibility (98 – 100% mortality); Possibility of resistance (90 – 97%); Resistance (<90%)

3.3.2 Genotypic resistance

A total of 1,400 *An. gambiae* s.l. were shipped to CREC for detection of the *Kdr* West and *Ace*-1R mutations.

Determinations of the *Kdr* L1014F (*Kdr* West) and *Ace*-1 mutations were carried out following the protocol of Martinez-Torres et al (1998)[14] and Weill et al (2004)[15] respectively.

Of 1,410 mosquitoes analyzed for *Kdr* PCR in all sites, 987 were resistant homozygotes (RR), 278 heterozygotes (RS), and 145 susceptible homozygotes (SS). The allelic frequency of the *Kdr* (F(Kdr)) mutation in *An. gambiae* s.s. ranged from 69% in Labé to 87 % in Kissidougou.

Furthermore, *Ace*-1R mutation was recorded in all localities with very low frequencies (F(Ace-1)), ranging from 1% in Siguiri to 5% in Kissidougou.

[14] Martinez-Torres. D., Chandre, F., Williamson, M. S., Darriet, F., Bergé, J. B., Devonshire, A. L., Guillet, P., Pasteur, N., and Pauron, D (1998) Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s. Insect Molecular Biology, 7*, 179–84.

[15] Weill, M., Malcolm, C., Chandre, F., Mogensen, K., Berthomieu, A., Marguine, M., and Raymond, M (2004) The unique mutation in Ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Molecular Biology, 13,* 1–7.

The detection results for *Kdr* West and *Ace*-1R mutations in *An. gambiae* s.l. are presented in ***Table 15***

Table 15: Summary of *Kdr* West and *Ace*-1R frequency distribution in *An. gambiae* s.l.

Sites	N tested	*kdr*				*Ace-1*			
		RR	RS	SS	F (Kdr)	RR	RS	SS	F (Ace-1)
Dabola	197	150	34	13	0.85	0	15	182	0.04
Faranah 2	115	83	20	12	0.81	0	7	108	0.03
Kankan	206	156	36	14	0.84	0	10	196	0.02
Kissidougou	197	157	28	12	0.87	0	19	178	0.05
Labé	198	115	44	39	0.69	0	9	189	0.02
Siguiri	198	134	43	21	0.79	0	4	194	0.01
Faranah 1	101	69	27	5	0.82	0	7	94	0.03
Lola	198	123	46	29	0.74	0	10	188	0.03
Total	**1,410**	**987**	**278**	**145**	**0.80**	**0**	**81**	**1329**	**0.03**

Annex. Laboratory Equipment, Materials, and Reagents

Entomological laboratory equipment and materials are summarized in ***Table A-1***.

Table A-1: Entomological laboratory equipment and materials

Goods	Amount	Comments
Generator	1	Good condition
Inverter 4000 and 5000 W	2	Good condition
Spectrophotometer calibration from 450 to 630 nanometers	1	Good condition
Desktop computer with accessories	1	Good condition
Stabilizer and two wall thermometers	1	Good condition
Refrigerator (for the cold chain)	4	Good condition
Garbage bags and plastic waste	6	Good condition
Cat food	1	Good condition
Pair of rabbits and food	2	Good condition
Sanitary equipment kit	1	Good condition
Large bucket (60 liters)	2	Good condition
Small bowls	16	Good condition
Table	2	Good condition
Stools	6	Good condition
Rolling chairs	3	Good condition
Lab coats	14	Good condition
Loupe	4	Good condition
Microscope	1	Good condition
Electric centrifuge	1	Good condition
Electric Pipette	1	Good condition
Accumet XL 200 with accessories	1	Good condition
Fischer scientific Isotemp	2	Good condition
Hot pad for heating surface	1	Good condition
Millipore unit	1	Good condition
Giant sterile 50 pairs	5	Good condition

Goods	Amount	Comments
Face masks 20 pack	20	Good condition

The reagents are summarized in ***Table A-2***.

Table A-2: Laboratory reagents

Goods	Amount	Observation
Citric acid	1	Good condition
Casein cattle	1	Good condition
Sodium chloride ACS	1	Good condition
Bovine serum albumin BSA	1	Good condition
Ortotolidine	1	Good condition
Tween 20 700 ml	1	Good condition
NN dimethyl formamide / 250ml	1	Good condition
Igepal CA - 630/50 ml	1	Good condition
thimerosal 10g	1	Good condition
Phenol red sodium salt 5 g	1	Good condition
Hydrogen peroxide 100 ml	2	Good condition
Sulfuric acid 500ml	1	Good condition
Glycerol 1l	1	Good condition
Disodium phosphate (Na2HPO4) Aliquot	1	Good condition
Potassium chloride (KCl) 1kg	1	Good condition
KH2PO4 potassium phosphate	1	Good condition
PBS 8 tablets / Aliquot	1	Good condition
Sodium hydroxide (NaOH) / Aliquot	1	Good condition
Sterile water	1	Good condition
Capture antibody (Pf)	1	Good condition
Conjugated antibody (PF)	1	Good condition
Antigens (PF0 Positive Controls)	1	Good condition
Nunc plate (ELISA)	1	Good condition
Water DNase 1 liter	1	Good condition
Hydrochloric acid 500 ml	1	Good condition
Acetone 1 liter	1	Good condition
Methanol 1 liter 95 degree	2	Good condition
Soda (NaOH) em tablets 100	1	Good condition
Orthotolidine Aliquot	1	Good condition
Silica gel 5Kg1	1	Good condition