FINAL REPORT STUDY nº	C0015/1718/03/ET/0001

STUDY REPORT TEMPLATE: EFFICACY TESTING OF MOSQUITO REPELLENT

SPONSOR	TEST FACILITY
RIF-RAF S.L. C/Abat Oliva, 4 08203 – Sabadell Barcelona	*Nettare Bio Labs Pvt Ltd* *No.4C, 5th Floor, Gamma Building* *Sigma Tech Park* *White Field Phase 2* *Bangalore – 560066* *India*

STUDY DIRECTOR	*Md. Afaque Hussain*
DATE	***Starting date: 12/03/2018*** ***Experimental phase starting date: 12/03/2018*** ***Experimental phase completion date: 21/03/2018*** ***Study completion date: 21/03/2018***

SUMMARY

COMPILANCE STATEMENT

This study was performed by *Nettare Bio Labs Pvt Ltd* test facility under my direction (undersigned) according to the protocols based on the indications supplied by the following guidelines:

- Documentation issued the European Commission: Technical Notes for Guidance. PRODUCT TYPE 18 – INSECTICIDES, ACARICIDES AND PRODUCTS TO CONTROL OTHER ARTHROPODS and PRODUCT TYPE 19 – REPELLENTS AND ATTRACTANTS (only concerning arthropods). CA-Dec12-Doc.6.2.a – Final.
- USA EPA (USA Environment Protection Agency), EPA Product performance test guidelines OPPTS 810-3700: insect repellents to be applied on human skin, July 7, 2010.
- WHO Guidelines for efficacy testing of mosquito repellents for human skin (WHO/CDS/NTD/WHOPES/2009.4).

The results of this report completely and faithfully reflect the raw data generated by the Study.

Date: 21/03/2018

The study director

Md. Afaque Hussain

KEY PERSONNEL

SPONSOR	
RIF-RAF S.L. C/Abat Oliva, 4 08203 – Sabadell Barcelona	
STUDY DIRECTOR	**TEST FACILITY MANAGEMENT**
Md. Afaque Hussain *No.4C, 5th Floor, Gamma Building* *Sigma Tech Park* *White Field Phase 2* *Bangalore – 560066* *INDIA* e-mail: *afaque@nblabs.in*	***Dr. P. Gayatri Hela*** *No.4C, 5th Floor, Gamma Building* *Sigma Tech Park* *White Field Phase 2* *Bangalore – 560066* *INDIA* e-mail: *hela@nblabs.in*
QUALITY ASSURANCE UNIT	**ANALYSTS**
Reshma *No.4C, 5th Floor, gamma Building* *Sigma Tech Park* *White Field Phase 2* *Bangalore – 560066* *INDIA* e-mail: *reshma@nblabs.in*	***Kiran Kumar B. B.*** *No.4C, 5th Floor, Gamma Building* *Sigma Tech Park* *White Field Phase 2* *Bangalore – 560066* *INDIA* e-mail: *kiran@nblabs.in*

1. OBJECTIVE OF THE STUDY

The objective of the study was to evaluate repellence of impregnated Textile samples with SI Repel mosquito treatment against *Aedes aegypti* mosquitoes by using "Mouse feed method".

2. TEST ITEM

Name*:* SI Repel mosquito Textile sample

Sample batch: NA

Preparation date: NA

Receiving date: 11/01/2018

Test Facility ID: NBL

Samples received: SI Repel mosquito Textile Sample

Further information: The samples were handle with gloves.

3. BACKGROUND

This test is to measure the effectiveness of treated with SI Repel mosquito actives, to protect against biting insects. Results are shown for treated samples.

4. TEST SPECIFICATIONS

Technique: To compare the behavior of mosquitoes towards a blood – meal target, Swiss albino mouse

Species: *Aedes aegypti* mosquitoes

Nº Repetitions: 3 repetitions

N° Mosquitoes Per Repetitions: 100 mosquitoes

Assessment Frequency: One hour with treated sample

Evaluation Method: Percentage of efficacy

5. DEFINITIONS

Bite: This term refers to an insect penetrating skin with its mouthparts and ingesting blood.

Landing: This term refers to an insect that lands on the exposed area on the mouse, but does not probe or bite.

Repellence: This term refers to a lack of insect's landing and probing mouse skin where repellent has been applied.

Species: This term refers 5 days old, un-fed female *Aedes aegypti* mosquitoes.

6. PARAMETERS OF EVALUATION

The Efficacy Percentage is calculated according to the following formula:

$$\% \text{ Efficacy} = 100 - \frac{\text{Landing on Treated } \times 100}{\text{Landing on Control}}$$

The Efficacy Percentage indicates the reduction of the number of landings on the treated sample, at every assessment, compared to the number of landings registered on the control sample at the beginning of the repetition and is calculated at the end of each trial.

7. EXPERIMENTAL DETAILS

7.1. APPARATUS

Cage Size*:*

Cubic, 20,000 cm^3, rectangular, with 1 sleeved opening for inserting the subject's arm in the cage. Test chamber is made of net (walls and top) and white fabric (underneath).

Figure 1- Square test chamber.

Parameters:

The temperature is kept at 25°C to 27°C and the relative humidity 60% - 80%. An artificial source of illumination is used.

In each cage, a source of water is available.

Two Time zone- European time zone and Brazilian time zone

7.2. SPECIES: *Aedes aegypti*

Rearing Technique For Mosquitoes*:*

The larvae and pupae are reared under optimal conditions at 27 ± 2°C, relative humidity 80 ± 10%, and photoperiod 16 : 8 hours (light : dark). Feeding of adults is done by providing a cotton pad soaked with 10% sucrose solution. No blood meal is given before the test. Mosquitoes are starved for 12 hours before the test. The test insects are used for only one test, and they are incinerated after the test.

Number of Mosquitoes:

100 per cage per repetition.

7.3. SAMPLES

Storage in the laboratory:

Samples are stored in a cool, dry place, within a plastic bag.

8. PROCEDURE

8.1. MOSQUITOES USED

- The mosquitoes are 5 days old females *of Aedes aegypti.*
- The mosquito progenies are maintained in drain water in a cage under room temp (27 ± 2°C), ventilation and relative humidity (75%).
- The mosquitoes are starved from blood meal for 24 hours before testing and fed on sugar water. They should be eager to bite.
- 100 mosquitoes are used for trial.

8.2. REPELLENCE TEST

- The trial is conducted in square repellent test chamber (with a mosquito net as walls and a mosquito net top and white cloth underneath under lab conditions.
- A mouse is maintained in a tight net cage with eyes protected.
- The test sample is wrapped around the mouse and placed inside the testing chamber.
- The Time interval for each study is One hour (60 min). The number of mosquitoes landed on the target and on the material is recorded for one hour.
- The knockdown effect on the insects is also noted.
- Identical procedure is followed for various configurations.

9. RESULTS

Table 1: Summary of the result

Sl. No.	Sample type	Activity of Mosquito Observed					
		European Time zone			Brazilian Time Zone		
		Landings on mouse	Landings on sample	Knockdown effect	Landings on mouse	Landings on sample	Knockdown effect
1	Control	86	00	0	78	00	0
2	SI Repel mosquito Textile sample	04	00	0	07	00	0

Table 2: % Efficacy

Sl. No.	Sample type	% Efficacy			
		European Time zone		Brazilian Time Zone	
		Landings on mouse	Landings on sample	Landings on mouse	Landings on sample
1	SI Repel mosquito Textile sample	95.34%	100%	91.02%	100%

10. CONCLUSION

- **In European Time Zone- SI Repel mosquito Textile sample** has shown **very good repellent efficacy of 95.34% on mouse** and **excellent repellent effect of 100% efficacy on sample** compared with control.
- **In Brazilian Time Zone- SI Repel mosquito Textile Sample** has shown **very good repellent efficacy of 91.02% on mouse** and **excellent repellent effect of 100% efficacy on sample** compared with control.