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PROJECT SPONSOR	
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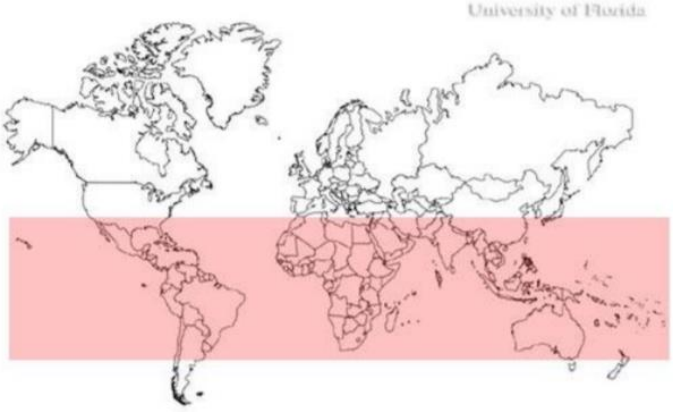
REF:	**Technical Report: Effect of using PCG ApS device referred to as the "Bug Bite Thing" on relief of itchiness and swelling after a single bite of the common house mosquito [*Culex quinquefasciatus*]**

Introduction

Commonly known as the southern house mosquito *Culex quinquefasciatus* Say is a medium-sized brown mosquito found widely in tropical regions and subtropical areas. This species is found throughout Florida and as far north as Virginia and Iowa and as far west as California. C. quinquefasciatus also breeds with the common house mosquito, *C. pipiens*. This hybridization results in a far larger range of territory. *C. quinquefasciatus* is an opportunistic blood feeder and a vector of many of pathogens, several of which affect humans. The Insect Laboratory of Poseidon Sciences Research & Development (Philippines) maintains a breeding facility for *C. quinquefasciatus* for research. Because this species is ubiquitous throughout the world, Poseidon Sciences have chosen this species as the primary model to study insect biology, behavior and repellents.



World distribution of the southern house mosquito, Culex quinquefasciatus Say. Illustration by Stephanie Hill, University of Florida.

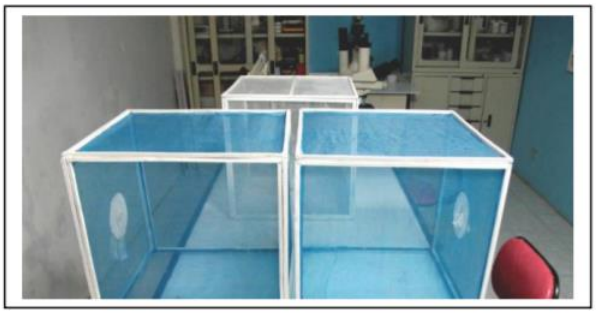
This report describes results derived from laboratory evaluation of samples received by Poseidon Sciences R&D from PCG ApS shown in the image below. The study evaluated the effectiveness of PCG ApS device called "Bug Bite Thing" on the response of human volunteers after a single bite of the *C. quinquefasciatus*. All studies were conducted at Poseidon Sciences Insect Control Laboratory (Miag-ao, Iloilo, Philippines). The tests were conducted over a period of three (3) weeks ending October 4, 2019.

Materials and Methods

All mosquito tests used the method previously described under the ASTM E951 (Standard test for the laboratory testing of non-commercial repellent formulations on the skin) and follow US EPA Guidelines for evaluation of repellents (US EPA Product Performance Test Guidelines (OPPTS 810.3700: Insect Repellents to be Applied to Human Skin). Copies of the standards are attached with this report.

Shown on the photo on the left were samples of the "Bug Bite Thing" used in this study. The device was shipped to Poseidon Sciences R&D (Miag-ao, Iloilo Province, Philippines) by PCG ApS The devices were kept at room temperature until used for the study.

A photograph of the laboratory test areas is shown below.

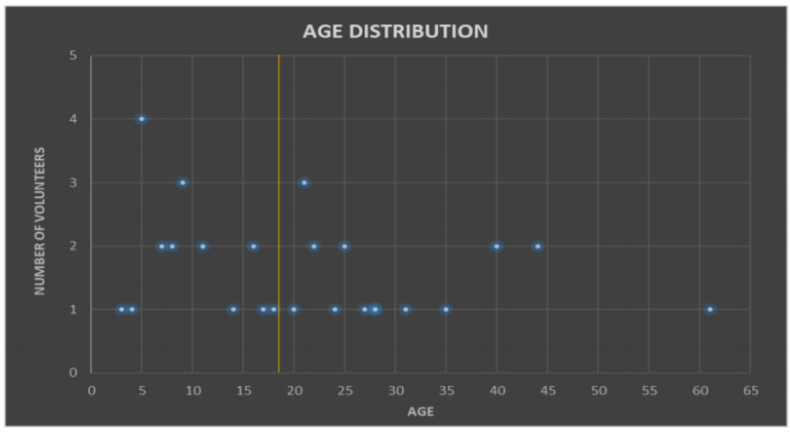


Test Subjects. A total of 40 human volunteers had been recruited for this study. All are Asiatic (Filipinos) from Miag-ao, Iloilo (Philippines). All are in good health, have not taken any medications (oral or topical) for at least 3 days before the study. Each volunteer had signed an Informed Consent Form. In the case of minors participating in this study, the parent/guardian signed the informed consent form and were present during the course of the test. This study had been approved by Poseidon Sciences IRB (Institutional Review Board).

The summary of age and sex of all the volunteers are shown in Table I below.

TABLE I. Age and Sex distribution of human volunteers in this study

GROUP	Total number of volunteers	Age range (years)	Mean Age ± SD ± SEM	Number of Males	Number of Females
MINORS	20	3-17	**8.7 ± 4.3 ± 1.0**	3	17
ADULTS	20	18-61	**29.9 ± 11 ± 2.4**	11	9
TOTAL	40	3-61		15	25



MINORS **ADULTS**

The mean age of minors (below 18 years) was 8.7 years of age, while adults was 29.9 years. All subjects did not consume any food for a period of at least 2 hours prior to the test and did not use any cosmetic product for at least 24 hours prior to the tests. There were some volunteers who were eliminated from the study. This occurred when the subject did not feel the bite of the mosquito or did not show any obvious marks or did not feel any itch from the bite. These represented only 3 subjects in the adult group and 5 in the minors group. In the case of minors, there are those in the younger age group that would refuse to insert their arm inside the cage or cry. These subjects are eliminated from the project before the start or prior to using the "Bug Bite Thing."

The Experimental Group represented the right forearm where the "Bug Bite Thing" was used. The Control Group comprised the same volunteers using the left forearm that did not receive any treatment. This study used the same individual as the experimental and control.

The study was conducted at room temperature (25° C to 28 °C) under ambient light conditions.

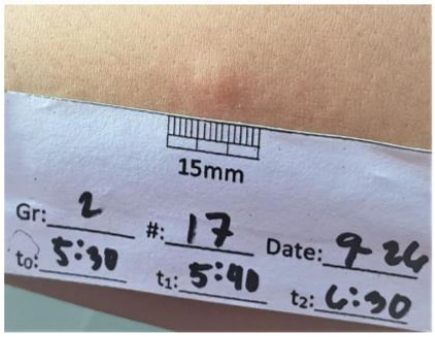
Procedure. The volunteer was made to wear a white cloth band that fitted from elbow to the wrist. An opening on the white cloth exposed a 30 cm^2 part of the underside of the forearm. The subject was made to wear a plastic glove to protect the hand and fingers from bites. The only area available for mosquitoes to bite was the exposed part of the forearm. The volunteer inserted the arm inside the cage containing unfed female mosquitoes until the first bite occurs. The volunteer was made to withdraw the arm outside the cage and immediately examined, photographed and then the "Bug Bite Thing" was used as described in the PCG ApS instruction for use. Typically, the volunteer would scratch the bite area soon after withdrawal from the cage and was allowed during this study only at this initial withdrawal. Further scratching was not permitted until completion of the evaluations.

The Control followed the same procedure as above except that the "Bug Bite Thing" was not used.

Evaluations The research scientist records the time of the bite. The evaluations were conducted by a subjective method through a Rating system described below. The research scientist asks the volunteer to rate the degree of itch and swelling. The timeline of testing and photography are as follows:

T_0 = time zero when observation and photography were made of the bite area. This was followed with the use of the "Bug Bite Thing" only once. The time elapsed during this period range from 20 seconds to 30 seconds.
T_5 = at 5 minutes after the "Bug Bite Thing" was used
T_{10} = at 10 minutes
T_{30} = at 30 minutes
T_{60} = at 60 minutes
T_{120} = at 120 minutes

Photographs were taken at various period between the time points t=0 to t=60 with a millimeter reference values and identification number of the volunteers. In young children, typically below 11 years of age, photography can be challenging because of movement of the arm. A sample of a typical photography is shown here for reference.



Rating System.

The investigator asked each volunteer to rate their perception of itchiness using a rating system shown below. For children below 10 years old, the parent or guardian served as intermediary to ask the child about how they felt and that perception was translated to the investigator.

For Itchiness:

1 = No itchiness
2 = Mild itchiness
3 = Itchy
4 = Very itchy

For Swelling/redness:

1 = No swelling/redness
2 = small swelling/redness
3 = moderate swelling/redness
4 = large swelling/redness
5 = very large swelling/redness

Perception of swelling and redness was a visual evaluation. Since each volunteer had no previous experience in evaluating this reaction, the evaluation was conducted by two experienced investigators who decided together the degree of the reaction.

Statistics. The raw data of each test group is provided in tabular form in Appendix I of this report. As a matter of policy, we prefer the project sponsor to decide on the appropriate statistical method to use. From time to time and as part of the discussion, we may use Student's t-test compare the significance between groups. A difference less than $P < 0.05$ is considered significant.

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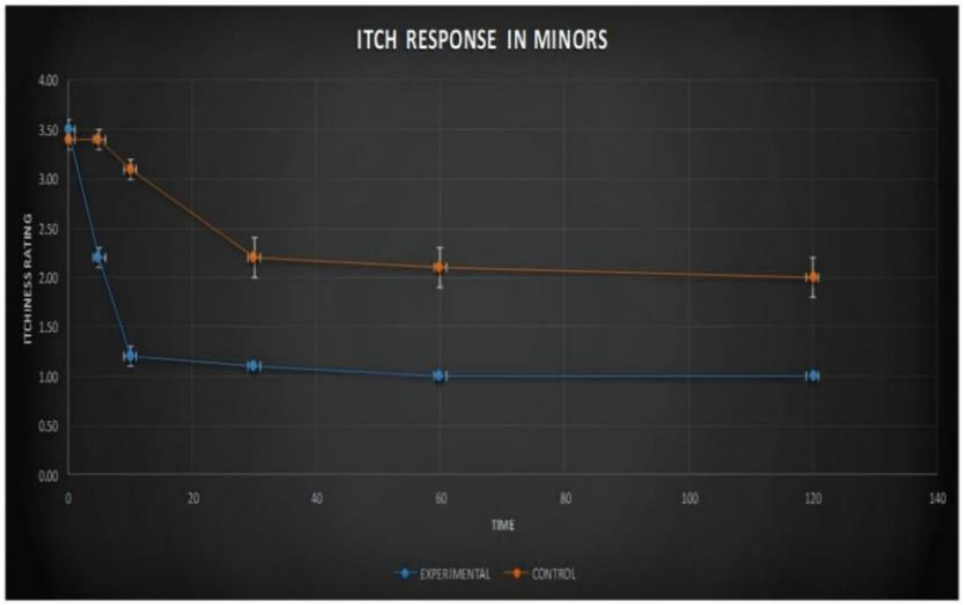
Results and Discussion

The data are presented in tabular format showing the protection time (minutes) for each human volunteer, with the mean, standard deviation (SD) and standard error of the mean (SEM) for each evaluation time point for each test group. The raw data are shown in Table I and II in Appendix 1 for reference.



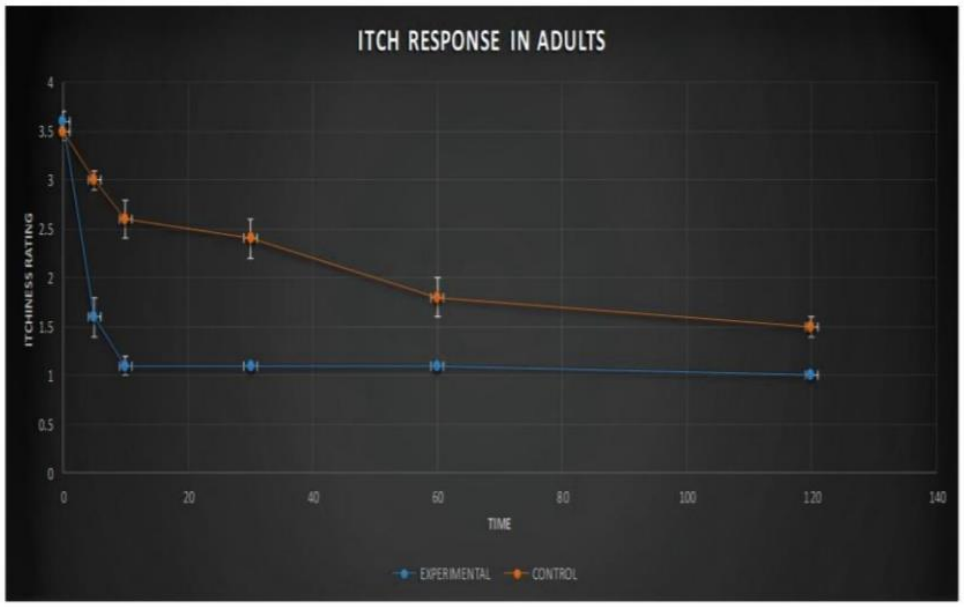


Figure 1. The response of minors and adults to the use of the "Bug Bite Thing" using the Itchiness Rating. Data are shown as mean ± standard error of the mean.

The results show that within 5 minutes after using the "Bug Bite Thing" the perception of itch vanished in both minors and adults. By 10 minutes, the volunteers on the experimental group no longer experience any itch. In contrast, the Control Group experienced continued itch which lasted for 1 hour after the bite occurred.

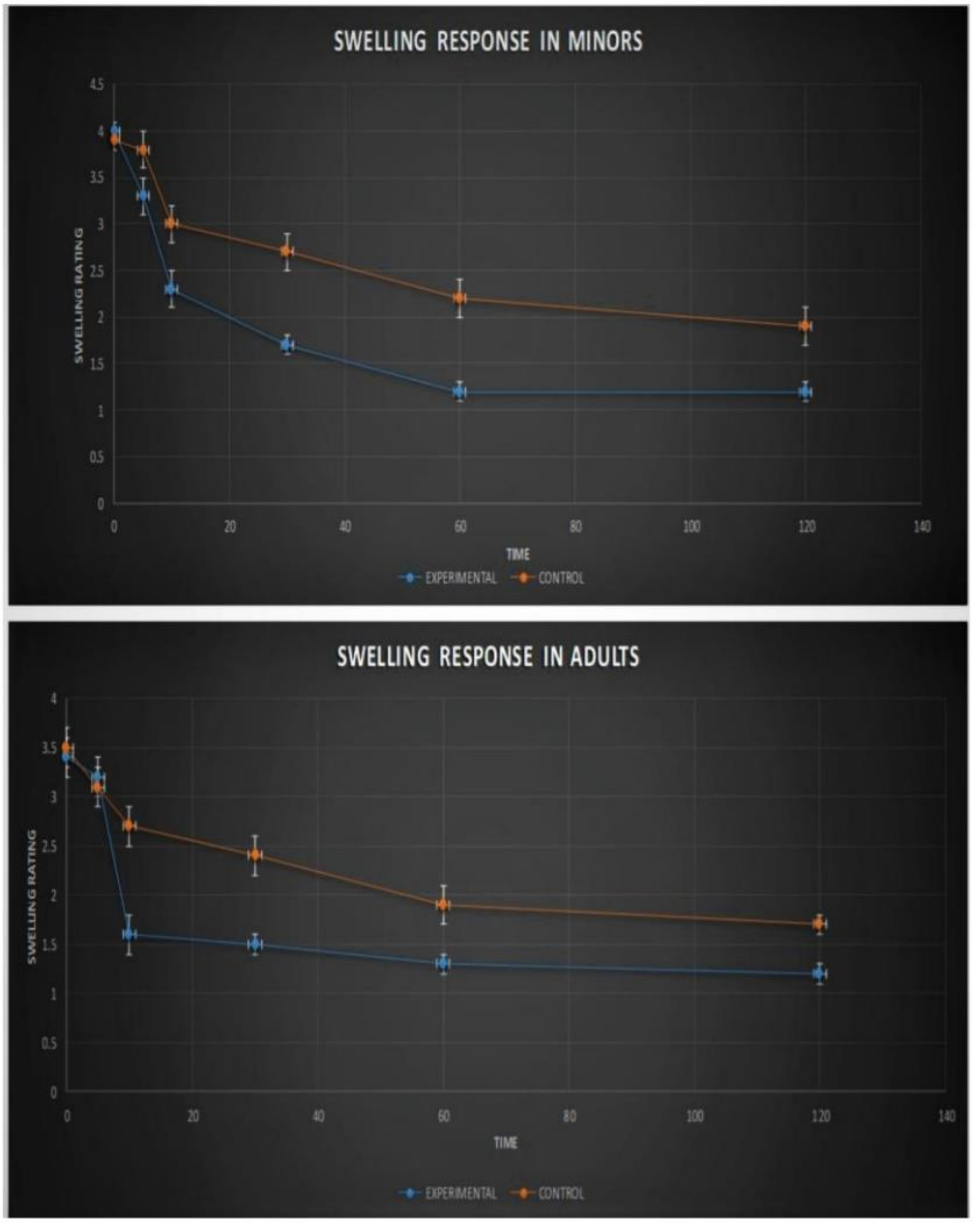
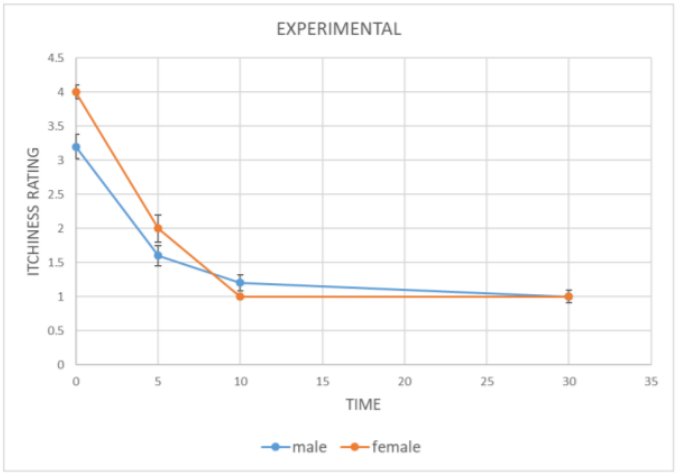


Figure 2. The swelling pattern in minors and adults to the use of the "Bug Bite Thing." Data are shown as mean ± standard error of the mean

Swelling and redness normally took time to fade after a mosquito bite. In the Control, it normally took over an hour to 2 hours to completely vanish. When the "Bug Bite Thing" was used the swelling and redness dramatically declined within 30 minutes, although a mild swelling and redness persisted until the termination of the study.

For all of the volunteers in the Experimental Group, there was a positive feeling of immediate relief from the itch, which was a primary concern with mosquito bites. The redness/swelling was considered a secondary issue and a minor concern for all the volunteers.

The data sets also allowed to answer the question whether there are sex differences in the response of the volunteers to the use of the "Bug Bite Thing." Figure 3 shows that the pattern is the same for both sexes for adults in terms of the Itchiness Rating in the experimental arm. When the untreated arm in the control group was subjected to the same evaluation, there was also no sex difference that are apparent. This evaluation was not conducted on minors since there was uneven number of males (3) and females (17) in the study.



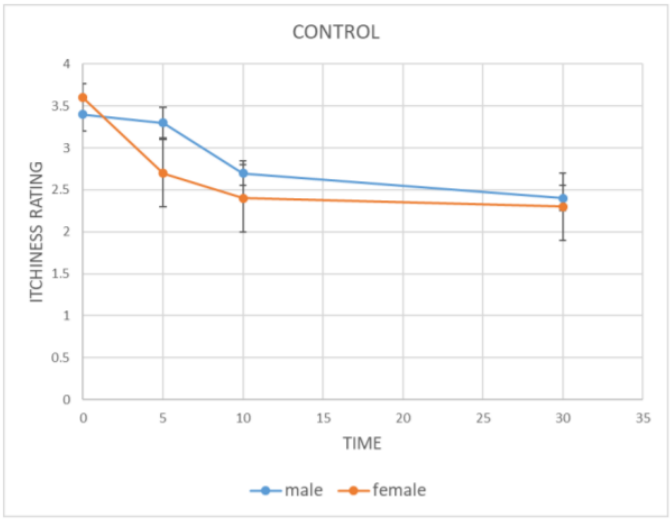


Figure 3. Comparison of the itch response of adult males and females to the use of the "Bug Bite Thing." Data are based on 11 males and 9 females; mean ± standard error of the mean

There is also the question of possible differences in the use of the "Bug Bite Thing" between minors and adults. The data on Figure 4 represent a comparison of the itch and swelling responses of minors and adults demonstrating that there were no age differences in responses.

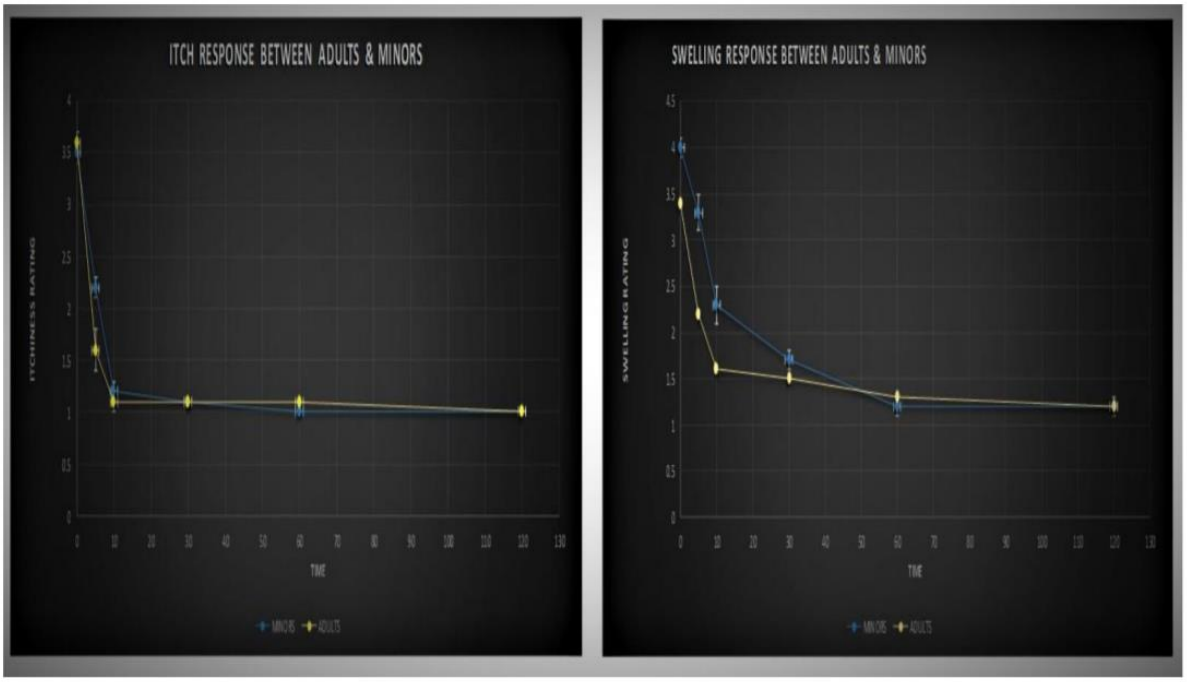


Figure 4. Comparison of the itch and swelling responses of minors and adults to the use of the "Bug Bite Thing." Mean ± SEM.

Photographic records and quantitative measurements.

Swelling and irritation are normally quite difficult to quantitatively measure. As an example, in the paper published in Clinical Transl Allergy, 2016 [Feb 23;6:8], measurement of the diameter of the irritation was found to be an inaccurate method of evaluation. The edges of the swelling and the redness could not be accurately determined and often subject to individual biases. Here is a typical example of a skin reaction where the swelling could not be easily delineated visually and photographically. Also, the gradation of colors is so slight that the use of color comparator to rate the intensity of coloration would not be accurate. The Filipino skin color are generally light brown to dark brown which makes edge identification of redness more difficult.

It is therefore not possible to quantitatively measure accurately the area or diameter of the redness or swelling associated with the mosquito bite.

Fig. 5 shows an example of the typical resolution of the swelling in a single individual followed by photography in both right and left forearm. The swelling in the control forearm remained for 30 minutes while in the forearm treated with "Bug Bite Thing" the swelling had already been reduced at 5 minutes and virtually gone by 30 minutes.

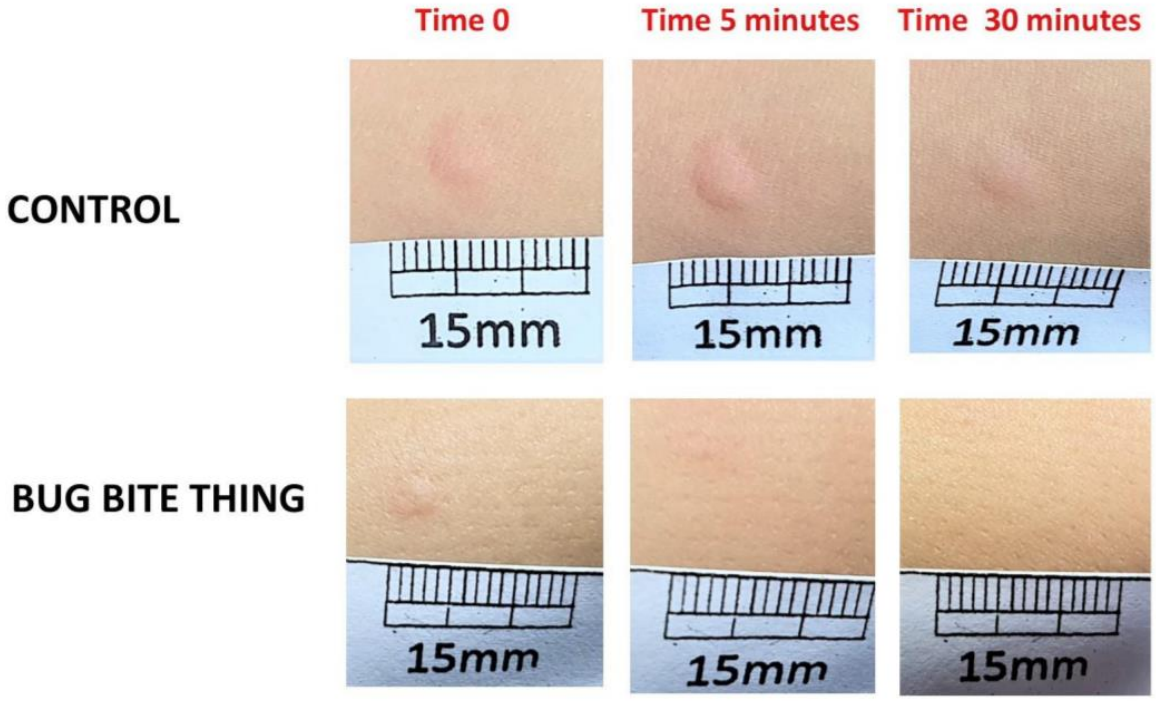


Figure 5. A series of photographs of single individual (age 17) demonstrating the process of resolution of the swelling and redness associated with mosquito bite.

Additional observations

Regarding the ease of use of the product, all the volunteers and investigators found the device easy to use on the flat surfaces of the forearm. During our preliminary trials, bites on fingers, elbows and ridges of bone on the legs tend to be difficult areas to use the larger aperture part of the device. Inverting the cap did help, but harder to get the suction on the right position of the bite area. Children did not have any particular problem when using the "Bug Bite Thing" on their arm.

24 hour Post-treatment evaluations.

All volunteers were interviewed 24 hours after the test to determine if there were any particular comments or reaction from the use of the "Bug Bite Thing." There were no reactions or negative comments associated with the use of the device.

CONCLUSION

The results clearly demonstrated that the "Bug Bite Thing" is an effective device to eliminate the itch associated with the bite of the mosquito, *Culex quinquefasciatus*. The effect is rapid and the relief can be felt as early as 1 minute after the use of the device. By 10 minutes, the vast majority of the volunteers felt no itching sensation. The relief from the redness and swelling took more time to resolve, but clearly much more reduced in intensity when the "Bug Bite Thing" was used.

The "Bug Bite Thing" is a revolutionary, safe and simple, nonchemical method to relieve the itch and inflammation associated with mosquito bites.

Reviewed and approved for submission to PCG ApS on October 10, 2019

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