



**PROJECT NO**  
421-0033

**STUDY TITLE**  
EVALUATION OF THE EFFICACY OF A ZIP-N-CLICK® ON PILLOW AND MATTRESS  
CASINGS FOR PREVENTION OF BED BUG ESCAPES

**IN-LIFE COMPLETION DATE**  
January 26, 2012

**STUDY COORDINATOR**  
Alicia Kelley

**PERFORMED FOR**  
Clean Brands, LLC  
400 Massasoit Ave  
East Providence, RI 02914-2012

**PERFORMED BY**  
ICR, Inc.  
1330 Dillon Heights Avenue  
Baltimore, MD 21228



## EXECUTIVE SUMMARY

The Zip-N-Click® on the Clean Brands pillow and mattress casings was tested for its ability to prevent bed bug escapes. Ten first instar bed bugs were placed in six 16 oz. plastic containers. The tops of the containers were covered with sections of the test fabric with the Zip-N-Click® zipper. The fabric sections were sealed to the outside of the containers with tape such that the only way for the bed bugs to escape was through the zippers. A heating pad (set to low: 31 – 32 °C) and a bed bug-scented piece of filter paper were placed outside the zipper as enticements for the bed bugs to escape. The three test containers had their zippers firmly closed. The three control containers had the zippers open. The containers were left overnight (14 hours). Observations of bed bugs were made the following morning.

None of the bed bugs in the test containers escaped, whereas an average of 53% of the bed bugs in the control containers escaped.

Alicia Kelley 1/31/12  
Alicia Kelley                      Date  
Study Coordinator



## TABLE OF CONTENTS

OBJECTIVE .....	4
MATERIALS.....	4
METHODS .....	4
RESULTS .....	5
CONCLUSION.....	5
APPENDIX I: PROTOCOL .....	7
APPENDIX I: RAW DATA SHEETS .....	12

## OBJECTIVE

To test the ability of the Zip-N-Click® on the mattress and pillow casings in preventing bed bug escapes.

This is not a GLP (Good Laboratory Practices, as defined by 40 CFR part 160) report.

## MATERIALS AND METHODS

The materials and methods were as described in protocol N4210112033A410 (APPENDIX I). A layout of the test arena is shown in figure 1. The bed bugs were placed in the plastic container and had to escape through the double zipper casing secured to its rim to reach the heating pad and filter paper scented with bed bug odor (Fig. 1 & 2).



Figure 1. Test Layout

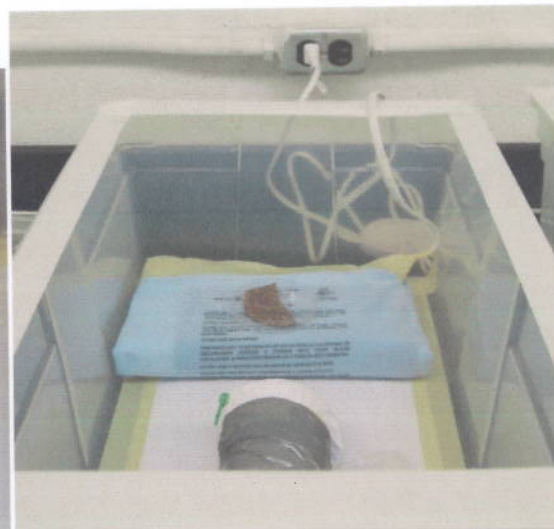


Figure 2. Test Arena

## RESULTS

None of the bed bugs in any of the three test containers escaped through the Zip-N-Click® zipper of the Clean Brands mattress and pillow casings. On average, 53% of the bed bugs in the control containers crawled through the open zippers (Table 1).

Table 1. Percent escapes for control and test replicates.

Rep	Percent Escaped	
	Control	Test
1	60%	0%
2	40%	0%
3	60%	0%
Average	53%	0%

## CONCLUSIONS

The Zip-N-Click® zipper used in the Clean Brands mattress and pillow casings was effective in preventing first instar bed bugs from escaping.



**APPENDIX I: PROTOCOL**



**PROTOCOL NO:**  
N4210112033A410

**PROJECT NO:**  
421-0033

**PROTOCOL TITLE:**  
EVALUATION OF THE EFFICACY OF A ZIP-N-CLICK® ON PILLOW AND MATTRESS  
CASINGS FOR PREVENTION OF BED BUG ESCAPES

**PROTOCOL VERSION DATE:**  
January 23, 2012

**PROPOSED START DATE:**  
January 2012

**PROPOSED COMPLETION DATE:**  
January 2012

**STUDY COORDINATOR:**  
Alicia Kelley

**PERFORMED FOR:**  
CleanBrands, LLC  
400 Massasoit Ave  
East Providence, RI 02914-2012

**PERFORMED BY:**  
ICR, Inc.  
1330 Dillon Heights Avenue  
Baltimore, MD 21228



### OBJECTIVE:

To test the ability of a Zip-N-Click® on pillow and mattress casings in preventing bed bug escapes.

This is not a GLP (Good Laboratory Practices, as defined by 40 CFR part 160) protocol, and the final report is not intended to be submitted to any regulatory agency as part of a GLP study or to support product registration.

#### *USE OF ICR'S NAME IN PROMOTIONAL RELEASES*

*Sponsor agrees not to use ICR's name in any promotional literature, TV, radio, web-based or other media, without the express written permission of ICR management. ICR, Inc. reserves the right to grant or deny this permission in its sole judgment based on the relation of the promotional text and images to the data generated by ICR for the sponsor.*

### MATERIALS:

- TEST FABRIC:** The fabric with Zip-N-Click® used in this test will be supplied by CleanBrands LLC.
- TEST ORGANISMS:** First instar bed bugs from the ICR colony (*Cimex lectularius*) reared at ambient indoor temperatures and humidity. This colony was obtained from the USDA Gainesville lab in July 1983.
- CONTAINERS:** 16 oz. plastic jars 4 in. high x 3.5 in. in diameter (bed bug rearing containers).
- ARENAS:** Plastic container (19.5 x 13.5 x 8.5 inches high)
- MISCELLANEOUS:** Forceps, rubber bands/tape, source of CO<sub>2</sub>, double sided tape, scotch tape, carpet tape, fluon, heating pad, petri dish, old bed bug filter paper.



## METHODS:

### *Summary*

Ten first instar bed bugs will be placed in a 16 oz. plastic container. The top of the container will be covered with the section of the test fabric where one zipper starts and the other zipper ends. The fabric will be sealed to the outside of the container with tape such that the only way for the bed bugs to escape will be through both zippers. The container will be laid on its side with a heating pad set to low (31 – 32 °C) positioned at its mouth. A petri dish containing a bed bug-scented piece of filter paper will be placed on the heating pad near the test container as an enticement for the bed bugs to escape. The container and heating pad will be placed in an arena. Three replications will be prepared with test fabrics where both zippers are closed. Three additional replications will be prepared to act as controls where both zippers are opened. The arenas will be allowed to set overnight (14 hours) under ambient temperature, humidity and lighting and observed for escapes the following morning.

### *Arena Preparation*

To help prevent any bed bugs from escaping, the lower inside walls of the arenas will be covered with fluon; the upper inside walls will be wrapped with double sided carpet tape. Double sided carpet tape will also be wrapped around the heating pad cord where it exits the arena.

### *Fabric Handling and Storage*

The fabric with Zip-N-Click® will be logged in when received and stored in a locked cabinet at ambient temperature and humidity until the study date.

### *Replication*

Three replications of test Zip-N-Click® and control Zip-N-Click® will be tested.

### *Handling of Bed Bugs*

The bed bugs will not have received a blood meal within 2 weeks prior to the test to ensure that they are hungry. Bed bugs will be anesthetized with CO<sub>2</sub> and then placed in vials in groups of ten until used in the test.

## DATA ANALYSIS:

The numbers of bed bugs escaping through the double zipper of the test fabric will be compared to the numbers escaping in the controls. Any bed bugs which escape from the test containers will constitute product failure.



CleanBrands, LLC  
Bed Bug Escape Test  
ICR Protocol No N4210112033A410  
ICR Project No. 421-0033

**SCHEDULE OF EVENTS**

<u>DATE</u>	<u>PROCEDURE</u>
Time Zero	Test Conducted
At End of Test	Telephone/email Report
Within 3 Weeks of Test	Written Report
Within 30 Days of Report	Fabric Returned

**STATEMENT OF AMENDMENT OR DEVIATION**

Any amendment to this protocol must be discussed with and approved by the sponsor. All amendments to, or deviations from, this protocol will be documented in the final report.

Alicia Kelley 1/25/12  
 \_\_\_\_\_  
 Alicia Kelley Date  
 Study Coordinator  
 ICR, Inc.

Irene Millette 1/24/12  
 \_\_\_\_\_  
 Irene Millette Date  
 CleanBrands, LLC



EVALUATION OF THE EFFICACY OF ZIP-N-CLICK® ON A PILLOW CASING IN PREVENTING BED BUG ESCAPES

Bed bug Strain: ICR Lab  
 1<sup>st</sup> instar bed bug nymphs per replicate: 10

Control: Open Zippers            or            Treated: Closed Zippers (circle one)

Date/Time of bed bug release:            Temp:    RH:

Date/Time of data collection:            Temp:    RH:

Rep	Confined*	Escaped**
<u>  1  </u>	_____	_____
<u>  2  </u>	_____	_____
<u>  3  </u>	_____	_____
<u>Total</u>	_____	_____
<u>Mean</u>	_____	_____

\*Did not get through zippers

\*\*Escaped through zippers into the arena

Recorder's Initials/Date:

Study Coordinator's Signature/Date



**APPENDIX II: RAW DATA SHEETS**



EVALUATION OF THE EFFICACY OF ZIP-N-CLICK® ON A PILLOW CASING IN PREVENTING BED BUG ESCAPES

Bed bug Strain: ICR Lab  
1<sup>st</sup> instar bed bug nymphs per replicate: 10

Control: Open Zippers or Treated: Closed Zippers (circle one)

Date/Time of bed bug release: 1/25/12 @ 5:00 pm Temp: 78° RH: 34%

Date/Time of data collection: 1/26/12 @ 7:00 am Temp: 77° RH: 35%

Rep	Confined*	Escaped**
<u>1</u>	<u>4</u>	<u>6</u>
<u>2</u>	<u>6</u>	<u>4</u>
<u>3</u>	<u>4</u>	<u>6</u>
<u>Total</u>	<u>14</u>	<u>16</u>
<u>Mean</u>	<u>4.7</u>	<u>5.3</u>

\*Did not get through zippers

\*\*Escaped through zippers into the arena

Recorder's Initials/Date: AK 1/26/12

Study Coordinator's Signature/Date Alicia Kelley 1/24/12



EVALUATION OF THE EFFICACY OF ZIP-N-CLICK® ON A PILLOW CASING IN PREVENTING BED BUG ESCAPES

Bed bug Strain: ICR Lab  
 1<sup>st</sup> instar bed bug nymphs per replicate: 10

Control: Open Zippers or Treated: Closed Zippers (circle one)

Date/Time of bed bug release: 1/25/12 @ 5:00 pm Temp: 78° RH: 34%

Date/Time of data collection: 1/26/12 @ 7:00 am Temp: 77° RH: 35%

Rep	Confined*	Escaped**
<u>1</u>	<u>10</u>	<u>0</u>
<u>2</u>	<u>10</u>	<u>0</u>
<u>3</u>	<u>10</u>	<u>0</u>
<u>Total</u>	<u>30</u>	<u>0</u>
<u>Mean</u>	<u>10</u>	<u>0</u>

\*Did not get through zippers

\*\*Escaped through zippers into the arena

Recorder's Initials/Date: AK 1/26/12

Study Coordinator's Signature/Date: Alicia Kelley 1/26/12