PROJECT NUMBER
421-0034

STUDY TITLE
EVALUATION OF THE EFFICACY OF 'CLEANBRANDS MATTRESS AND PILLOW ENCASEMENT FABRIC - BED BUG BITE PROOF' FOR PREVENTION OF BITING BY BED BUGS

PROTOCOL NUMBER
N4210212034A333

IN-LIFE COMPLETION DATE
March 21, 2012

STUDY COORDINATOR
Alicia Kelley

PERFORMED FOR
CleanBrands, LLC
400 Massasoit Ave, Ste 300
East Providence, RI 02914-2012

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

EXECUTIVE SUMMARY

'Cleanbrands Mattress and Pillow Encasement – Bed Bug Bite Proof' was tested to determine if bed bugs could pierce the fabric and bite a human subject's forearm. Single adult bed bugs were placed in 9-dram vials. The mouths of the vials were covered with the test fabric. One such vial was inverted on the forearm of a human subject. The vials were held there until the bed bugs bit and blood ingestion was evident, or until five minutes had elapsed, whichever occurred first. If the bed bug did not bite, the procedure was repeated with the same insect, but the test fabric was replaced with a screen through which bed bugs can bite. If the bed bugs bit through this screen, it confirmed that the bed bugs were eager to bite and only the test fabric prevented it. This method of exposure was repeated until 10 confirmed-biting adult bed bugs and 10 confirmed-biting mid-instar bed bugs were tested.

None of the bed bugs tested were able to bite through 'Cleanbrands Mattress and Pillow Encasement – Bed Bug Bite Proof' fabric. We confirmed this observation with ten adult bed bugs and ten mid-instar bed bugs which had a confirmed bite once switched to a vial with a screen they could feed through.

Alicia Kelley Date
Study Coordinator

TABLE OF CONTENTS

OBJECTIVE

To test the ability of "CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof" to prevent bed bugs from biting human subjects, in a laboratory setting.

This is not a GLP (Good Laboratory Practices) study or protocol, and the final report is not intended to be submitted to any regulatory agency as part of a GLP study.

MATERIALS AND METHODS

The sponsor provided the following test sample:

1. CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof

The materials and methods were as described in protocol N4210212034A333 (APPENDIX I).

'Cleanbrands Mattress and Pillow Encasement – Bed Bug Bite Proof' was tested to determine if bed bugs could pierce through the fabric. Single adult bed bugs were placed in individual 9-dram vials. The vial was covered with the test fabric (Figure 2). One such vial was inverted on each forearm of the subject and held there until the bed bugs bit and blood ingestion was evident, or for five minutes, whichever occurred first (Figure 1). If the bed bug did not bite, the procedure was repeated with the same insect, but the test fabric was replaced with a screen through which bed bugs can bite (Figure 3). If the bed bugs bit through this screen, it confirmed that the bed bugs were eager to bite and only the test fabric prevented it. This method of exposure was repeated until 10 confirmed-biting adult bed bugs and 10 confirmed-biting mid-instar bed bugs were tested.

Figure 1: Vials containing one bed bug inverted on subject's arm

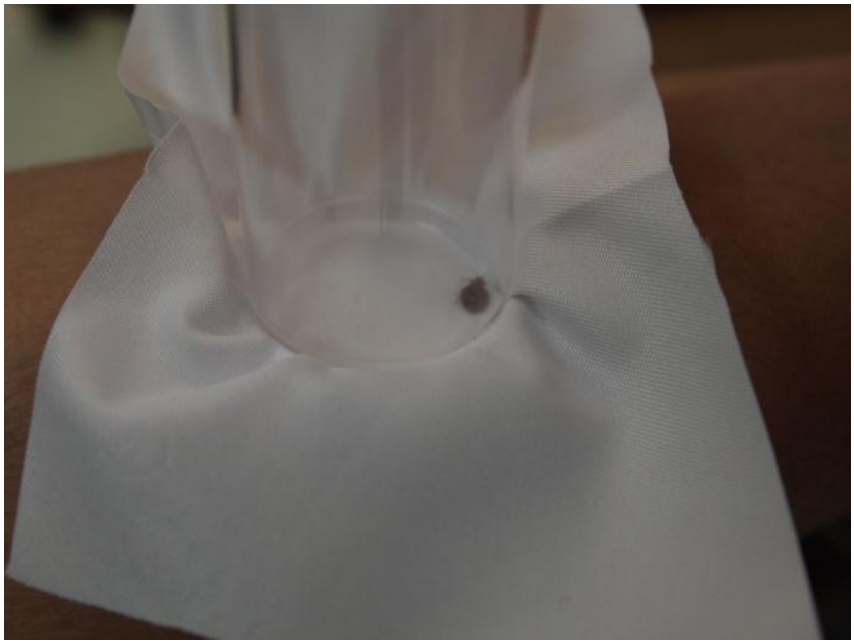

Figure 2: Vial with test fabric

Figure 3: Vial with control fabric

RESULTS

None of the 20 bed bugs tested were able to bite through the test fabric. All bed bugs tested which did not feed through the control fabric were discarded and a new bed bug was tested. These unconfirmed bed bugs were not recorded. This was repeated until ten confirmed biting bed bug adults and ten confirmed biting mid-instar bed bugs had been tested.

Adult Bed Bugs			**Mid-instar Nymphs**		
Replicate	Bitten through test fabric?	Bitten through control fabric?	Replicate	Bitten through test fabric?	Bitten through control fabric?
1	No	Yes	1	No	Yes
2	No	Yes	2	No	Yes
3	No	Yes	3	No	Yes
4	No	Yes	4	No	Yes
5	No	Yes	5	No	Yes
6	No	Yes	6	No	Yes
7	No	Yes	7	No	Yes
8	No	Yes	8	No	Yes
9	No	Yes	9	No	Yes
10	No	Yes	10	No	Yes

Table 1: Ten adults and ten nymphs which were unable to bite through test fabric were confirmed to bite through control fabric.

CONCLUSIONS

No bed bugs tested were able to pierce through the test fabric. 'CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof' was effective in preventing bed bugs from biting.

APPENDIX I: PROTOCOL

PROTOCOL NUMBER:
N4210212034A333

PROJECT NUMBER:
421-0034

STUDY TITLE:
EVALUATION OF THE EFFICACY OF 'CLEANBRANDS MATTRESS AND PILLOW ENCASEMENT FABRIC - BED BUG BITE PROOF' FOR PREVENTION OF BITING BY BED BUGS

PROTOCOL VERSION DATE:
March 7, 2012

PROPOSED START DATE:
March 2012

PROPOSED COMPLETION DATE:
March 2012

PRINCIPAL INVESTIGATOR:
Alicia Kelley

SPONSOR:
CleanBrands, LLC
400 Massasoit Ave, Ste 300
East Providence, RI 02914-2012

TESTING FACILITY:
ICR, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228-1199

OBJECTIVE:

To test the ability of "CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof" to prevent bed bugs from biting human subjects, in a laboratory setting.

This is not a GLP (Good Laboratory Practices) study or protocol, and the final report is not intended to be submitted to any regulatory agency as part of a GLP study.

MATERIALS:

TEST FABRIC:	The Sponsor will provide sample of "CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof." The sample will not contain any pesticides.
CONTROL SCREEN:	Fine screen used by ICR to maintain its bed bug colony; bed bugs easily can bite through this screen.
TEST ORGANISMS:	Second instar bed bugs and adult bed bugs from the ICR colony *(Cimex lectularius)* reared at ambient indoor temperature and humidity. They will have been starved for at least two weeks prior to this test.
HUMAN SUBJECT:	There will be one test subject used for this test. The subject will have both arms exposed to vials containing one bed bug. ICR, Inc. policy complies with the Department of Health and Human Services Policy at 45 C.F.R. pt. 46 and 45 C.F.R. §§ 46.109, 46.116, and the EPA Part 26 model rule at 40 C.F.R. pt. 26, when human volunteers are used. Thus ICR submits a protocol, informed consent document, MSDS or similar information of test substances, and standard indemnification forms to an independent institutional review board (IRB) set up to ensure that the rights and welfare of the participants are protected and that the study is carried out in an ethical manner. Insect Control & Research, Inc. uses the following IRB for this service: Independent Institutional Review Board Inc. 6738 West Sunrise Boulevard, Suite 102 Plantation, Florida 33313

Approval of all documentation for human volunteer testing must be obtained before such testing can be accomplished.

The subject will have signed "Informed Consent Documents" and completed a Test Subject Selection Checklist prior to being accepted as the study participant.

CONTAINERS: Glass vials (7 x 2 cm ext. diam.) with lids cut to leave only the threaded collars (these allow the fabric or screen to be secured over the vial mouths by screwing them down over it).

MISCELLANEOUS: Forceps, rubber bands/tape, source of CO_2, screen fabric

METHODS:

Summary
An adult bed bug will be placed in a 9-dram vial. The vial will be covered with the test fabric. Two such vials will be inverted on each forearm of the subject and held there until the bed bugs bite and blood ingestion is evident, or for five minutes, whichever occurs first. If a bed bug does not bite, the procedure will then be repeated with the same insect, but using a screen which they can bite through. If the bed bugs bite through this screen, it will confirm that the bed bugs were eager to bite and only the test fabric prevented it. This method of exposure will be repeated until 10 bed bugs have been tested.

Sample Handling and Storage
The sample will be logged in when received and stored in a locked cabinet at ambient temperature and humidity until the study date. The fabric samples will be cut as to provide sufficient swatches which are approximately 3 cm x 3 cm.

Replication
Ten replicates of one confirmed biting adult bed bug and 10 confirmed biting second-instar nymph will be tested.

Handling of Bed Bugs
The bed bugs will be selected from ICR's lab strain colony. The bed bugs will have been deprived of a blood meal for at least two weeks prior to the test to ensure they are hungry. The date of the last blood meal will be noted. Ten adult bed bugs will be anesthetized with CO_2 and then placed separate vials. The same procedure will be followed for ten early instar bed bugs.

Test Exposures
One bed bug will be tested on each arm of the subject simultaneously. ICR staff member will

invert two vials, each containing one bedbug, onto both forearms of the volunteer. The vials will be held in place with sufficient pressure to ensure contact of the fabric with the skin. The vials will be held in this manner for five minutes, or until the bed bugs bite and begin to ingest blood, (as evidenced by abdominal color change), whichever occurs first. Some individuals cannot feel bed bugs biting; therefore biting can often only be confirmed by observing the insects' blood-swollen abdomens. The bed bugs will be examined under a dissecting microscope if there is any doubt as to their having fed.

Control Exposures

Any bed bugs which do not bite through the test fabric will be given the opportunity to bite through screen which these insects are known to bite through. Control exposures will start within two hours of the last test exposure. The above procedure for test exposures will be repeated with these non-biting bed bugs, but the mouths of the vials will now be covered with sections of the control screen. If these bed bugs bite, it will confirm that they would have bitten through the test mattress cover, if only they had they been able to penetrate it.

Bed bugs which do not bite through the screen will be replaced with new ones. Testing will continue until 10 confirmed biting adults and 10 confirmed biting nymphs have been tested.

The exposures to bed bugs will go on for up to two (2) hours for each fabric. The study is expected to last no more than four (4) hours. These tests will all be done on one human subject.

Human Subject

There will be one test subject used for this test. He/She will sign an Informed Consent Agreement approved by an IRB prior to participation in the study. The subject will have both arms exposed to vials containing one bed bug. This will be repeated until a 10 adult bed bugs and 10 early-instar nymphs (20 total bed bugs) are tested on the subject.

Test subjects will be instructed to avoid alcohol, caffeine, nicotine, and fragrance products (e.g., soap, perfume, cologne, hair spray, lotion, antiperspirant/deodorant, etc.) 12 hours before and during the test. There will be no disease testing or alcohol/drug screening prior to this test.

Personnel preparation

At the onset of the study, the test subject will wash his/her forearms (wrist to elbows) with unscented Neutrogena® soap, then rinse with water and dry with a clean towel. There will be no other treatment.

Inclusion/Exclusion

The test subjects must appear to be in good health with no skin conditions.
The test subjects must be free of allergies, asthma, and eczema.
The test subjects must be free of significant skin disease, skin problems, a known sensitivity or allergy to insect bites, Elastikon (or equivalent) tape, latex, insect repellents, or other skin care products.
The test subjects must be free of taking any medication or of any concurrent illness which in the

opinion of the study coordinator may interfere with the study or its results.
It must be established that mosquitoes are attracted to the test subject's untreated skin.
The test subject must be willing to follow the study procedures as explained and be willing to sign an informed consent document.
The test subject must not be currently pregnant or a breast-feeding mother. An over-the-counter pregnancy test will be administered on-site to confirm pregnancy status.

Discomfort and Hazard
It is generally believed that bed bugs do not carry any diseases since there has been no conclusive evidence that they have done so. There are also no records of bed bugs transmitting disease. The bed bugs used in this test have been colonized at ICR since 1983, and have been kept in the laboratory, away from possible infection from infected people since then. A bite from one of these bed bugs may only be irritating, and will not transmit any diseases.

Reactions to bed bug bites vary greatly with the individual. Bed bug bites are painless, however some people develop an allergic reaction to the saliva the bed bug injects as it bites. If the subject reacts to the bite, it will probably become a red welt, the size of which varies with the individual. The welt may not appear for a few days after the bite occurs and will usually fade shortly after that. The irritation from the welt may itch, become red and/or swell. The skin of some individuals is more easily irritated than that of others. The irritation will usually disappear within a couple of days; however, some individuals may develop scarring or infection at the site of the bite. It is also possible to have a severe allergic reaction to the bite. In the case of a severe reaction, the test supervisor will refer him/her to a physician for treatment at ICR's expense. The subject may become sensitized to bed bug bites after repeated exposures. If this occurs, the subject will not be able to participate in future tests.

Monitoring of Study Conduct
Both the Study Coordinator (Principle Investigator) and Study Associates have reviewed the protocol and will conduct the study as per the protocol's specifications.

Confidentiality of Test Subjects
The information obtained from test subjects' taking part in this test will be used by ICR and its sponsor and will become part of a report. Only subject's initials will appear on data sheets. This report will be kept as confidential as possible under local, state and Federal law. ICR cannot guarantee that identities will be kept confidential. Independent IRB, Inc. has the right to review subject records.

Reporting Unanticipated Problems
Test subjects will be informed both verbally and in writing of any significant new findings discovered during the course of this study which may influence continued participation. These findings will also be reported, in writing, to the IIRB in a timely manner after its discovery.

Test Subject Participation and Withdrawal
Test subjects may refuse to take part in this study or quit at any time without penalty or loss of

benefits to which they may be otherwise entitled. Test subjects must tell the Study Coordinator (Principle Investigator) that they would like to drop out of the study; no written notification is needed. The Study Coordinator or sponsor may remove any subject that does not follow instructions given to them and agreed upon prior to the start of the study. If test subjects choose to drop out of the test, they will not be paid. If the sponsor and/or ICR asks test subjects to drop out of the test, and test subjects have complied with all requests, full payment will still be made. If ICR asks test subjects to drop out of the test because directions have not been followed, these test subjects will not be paid. The sponsor may stop the study or the test subject participation at any time, for any reason. If this occurs all subjects will be paid for the study. If any adverse effects are experienced by any subject, they will immediately be removed from the study and appropriate mediation will be taken.

Benefits to Subject

Test subjects will probably get no personal benefit from this study. The benefit to society is the development of a mattress covering that will prevent bed bug bites.

DATA ANALYSIS:

The numbers of bed bugs biting through the test fabric and through the screen will be compared. These data may be subjected to statistical analysis if appropriate. Any bedbug which is able to bite through the test fabric will be considered a product failure.

SCHEDULE OF EVENTS:

DATE	PROCEDURE
Day 0	In-life test conducted
After in-life test	Telephone/fax report
Within 30 days of in-life test	Written report
After written report	Unused sample returned

STATEMENT OF DEVIATION OR AMENDMENT

All amendments 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 Date
Study Coordinator
ICR, Inc.

Irene Millette Date
Product Manager
CleanBrands, LLC

BED BUG BITE RAW DATA SHEET

Test Date: **Date of Last Blood Meal:**

Test Fabric Name: CleanBrands Mattress and Pillow Encasement Fabric - Bed Bug Bite Proof

Bed Bug strain:

Life Stage: 2nd instar nymphs or Adults (Circle one)

Volunteer: Identified by code only to preserve privacy

Replicate (bed bug)	BITTEN THROUGH TEST FABRIC? (YES/NO)	BITTEN THROUGH CONTROL FABRIC? (YES/NO)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Subtotal		
Total		

Notes:

Recording Technician/Date:

APPENDIX II: RAW DATA SHEETS

Last Page (2 data sheets)