



Just Encase My Mattress, Inc.
Bed Bug Mattress Encasement Infestation Test
ICR Project No.: 459-0030
In-Life Completion Date: October 9, 2009

PROJECT NO:
459-0030

STUDY TITLE
EVALUATION OF A MINIATURE MATTRESS ENCASUREMENT IN PREVENTING
PENETRATION BY BED BUGS

PROTOCOL NO:
N45909090030A332
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IN-LIFE COMPLETION DATE:
October 9, 2009

STUDY COORDINATOR:
William Gaynor

PERFORMED FOR:
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Boca Raton FL 33433

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



EXECUTIVE SUMMARY

Foam blocks were inserted into three miniature mattress encasements. The encasements were then zippered shut. The encased 'mattresses' were placed in containers to prevent the escape of bed bugs. Twenty (10 adult and 10 1st instar) bed bugs were placed on each of the test mattress encasements. Each control replicate was subjected to these same procedures, except that the encasements were not sealed (the zipper was left open). Six days later, the numbers and locations of all bed bugs found were noted as inside or outside the mattress encasement. Any penetration of the mattress encasements (test and control) was presented as a percentage and the average percentage over three replicates was obtained. The results are shown below:

Treatment	Stage	% recovered inside
Control	Adults	40
	Nymphs	46.7
Ultraguard Mattress Protector	Adults	0
	Nymphs	0

No bed bugs were found inside the Ultraguard Mattress Protector encasements after six days however, bed bugs were found (40% of adults and 46.7% of nymphs) inside the control (open) encasements. These results provided strong evidence that Ultraguard Mattress Protector encasements prevent penetration of mattresses by bed bugs.

William Gaynor
Study Coordinator

10/23/09

Date:



TABLE OF CONTENTS

OBJECTIVE	4
MATERIALS & METHODS	4
RESULTS	4
CONCLUSIONS	7
APPENDIX I: PROTOCOL	8
APPENDIX II: SPREADSHEETS	14
APPENDIX III: RAW DATA SHEETS	16



OBJECTIVE:

To determine the efficacy of a mattress encasement against the infestation of a mattress by bed bugs.

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.

MATERIALS & METHODS:

The materials and methods used are as described in N45909090030A332 (Appendix I).

The sponsor provided the following samples:

1. Sample-sized mattresses (foam blocks)
2. Ultraguard Mattress Protectors (sample sized- mattress encasements).

There were two deviations:

1. Sample-sized mattresses sent by the sponsor were foam blocks instead of styrofoam blocks.
2. Mattresses were examined for infestation after six days rather than five days as specified in the protocol.

These deviations did not negatively impact the study.

RESULTS

Foam blocks were inserted into three miniature mattress encasements and sealed shut with their zipper. The encased 'mattresses' were placed in containers to prevent the escape of bed bugs. Twenty (10 adult and 10 1st instar) bed bugs were placed on each of the test mattress encasements. Each control replicate was subjected to these same procedures, except that the

encasements were not sealed (the zipper was left open). Six days later, the numbers and locations of all bed bugs found were noted as inside or outside the mattress encasement. Any penetration of the mattress encasements (test and control) was presented as a percentage and the average percentage over three replicates was obtained. The test-set-up is demonstrated below in Figures 1 and 2 and the results are shown in Table 1:

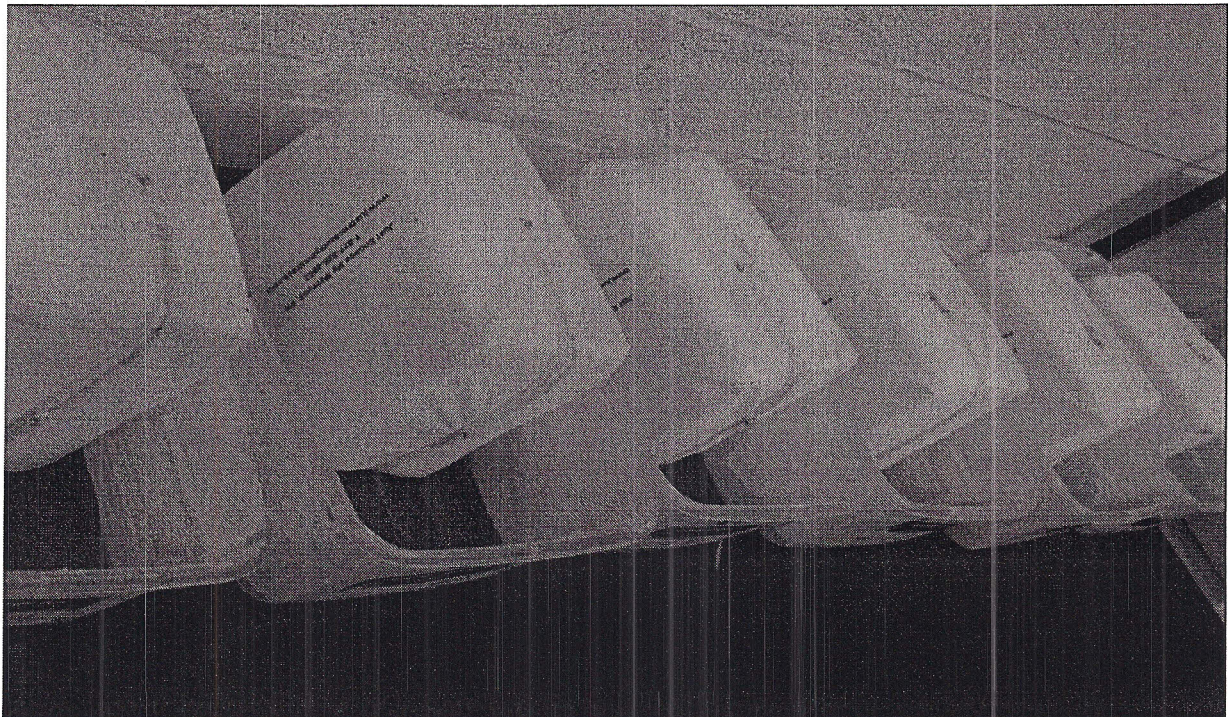


Figure 1. Test set-up showing three test encasements (closed zippers) and three control encasements (opened zippers).

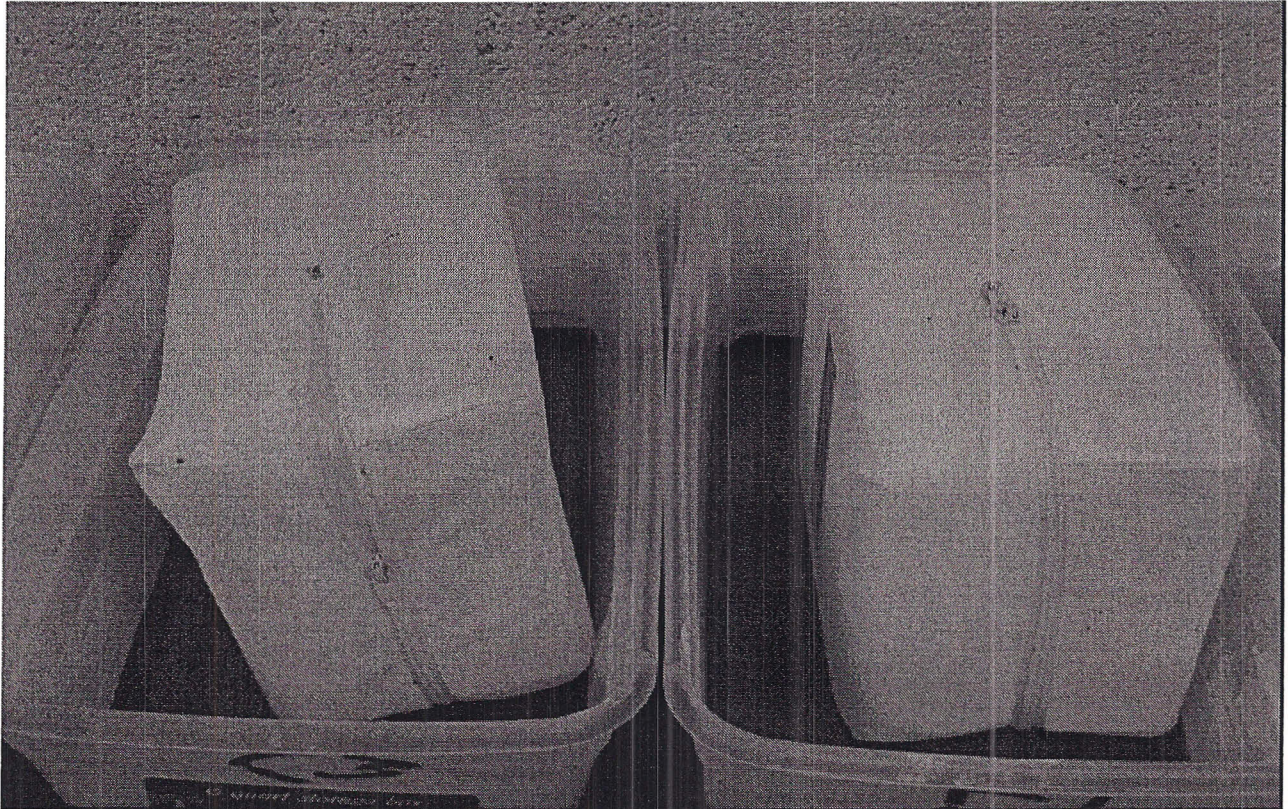


Figure 2. Close-up of one test encasement with the zipper closed (right) and one control encasement with the zipper left open (left).

Table 1. Average percentage of adult and nymphal bed bugs found inside either the control or test mattress encasement after 6 days.

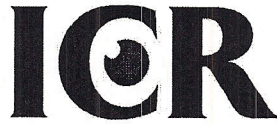
Treatment	Stage	% recovered inside
Control	Adults	40
	Nymphs	46.7
Ultraguard Mattress Protector	Adults	0
	Nymphs	0



When mattresses were covered with the Ultraguard Mattress Protector encasement, none of the bed bugs were found inside the encasements. However, 46.7% of the nymphs and 40% of the adult bed bugs were found inside the control (open) encasements.

CONCLUSIONS:

No bed bugs were found inside the Ultraguard Mattress Protector encasements after six days however, bed bugs were found (40% of adults and 46.7% of nymphs) inside the control (open) encasements. These results provided strong evidence that Ultraguard Mattress Protector encasements prevent penetration of mattresses by bed bugs.



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APPENDIX I: PROTOCOL



Just Encase My Mattress, Inc.
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Protocol No.: N45909090030A332
ICR Project No. 459-0030

PROTOCOL NO:
N45909090030A332
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PROJECT NO:
459-0030

PROTOCOL TITLE
EVALUATION OF A MINIATURE MATTRESS ENCASEMENT IN PREVENTING
PENETRATION BY BED BUGS

PROTOCOL VERSION DATE:
September 27, 2009

PROPOSED START DATE:
TBD

PROPOSED COMPLETION DATE:
TBD

STUDY COORDINATOR
William Gaynor

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OBJECTIVE:

To determine the efficacy of a mattress encasement against the infestation of a mattress by bed bugs.

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 this permission based on the relation of the promotional text and images to the data generated for the sponsor.

MATERIALS:

- TEST FABRIC:** The Sponsor will provide the sample-sized mattress and sample sized-mattress encasement as ready-to-use.
- TEST ORGANISMS:** Adult and 1st instar bed bugs (*Cimex lectularius*) reared at ICR will be used.. This colony was obtained for the USDA Gainesville lab in July 1983. The bed bugs will have been blood fed within one day of testing to ensure they are looking for some place to hide and digest their meal.
- CONTAINERS:** Large containers, measuring at least 54cm x 64cm. The inner walls may be lined with plastic and/or covered with Fluon® to prevent escapes.
- MISCELLANEOUS:** Forceps, source of CO₂, disposable latex gloves, deep freezer, blocks of Styrofoam.

METHODS:

Summary

Styrofoam blocks will be inserted into three miniature mattress encasements and sealed shut. The encased 'mattresses' will be placed in containers to prevent the escape of bed bugs. Twenty (10 adult and 10 1st instar) bed bugs will be placed on each of the test mattress encasements. Five days later, the numbers and locations of all bed bugs found will be noted as inside or outside the mattress encasement.

A set of controls will be run at the same time, with equal numbers of bed bugs.

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.

Replication

There will be three replicates of twenty (10 adult and 10 1st instar) test bed bugs and an equivalent number of control bed bugs.

Bed Bug Handling

Bed bugs will be anesthetized with CO₂ and picked for testing.

Test Exposures

Three styrofoam blocks will be used to simulate miniature mattresses (henceforth 'mattresses'). Each block will be covered by a mattress encasement. The encasements will be sealed as per manufacturers instructions (typically this involves zipping it tight). The test 'mattresses' and encasements will be placed into testing containers.. The bed bugs will be released on top of the mattress encasement where they will have free range for the duration of the test. This set-up will be maintained in the laboratory under ambient conditions with a ca. 15:9 light:dark cycle for 5 days.

At 5 days, the 'mattresses' and encasements will be examined and the bed bugs will be recovered. Their location upon recovery will be noted as "inside" the 'encasement' or "outside".

Control Exposures

Each control replicate will be subjected to the same procedures outlined above, except that the encasements will not be sealed (typically the zipper will be left open). The controls will be housed in the same area as those treated for the duration of the prescribed observation period.



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DATA ANALYSIS:

Any bed bugs found inside the test encasements will constitute failure of protection. If no bed bugs are found inside the test encasements, but bugs are found inside the control (open) encasements, this will be strong evidence for the encasements prevent penetration.

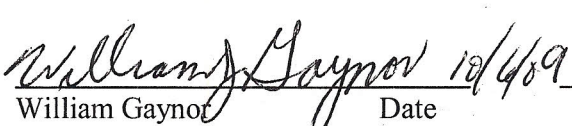
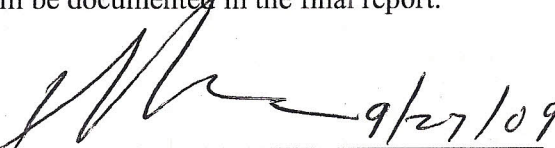
Any penetration of the mattress encasements (test and control) will be presented as percentages. Penetration of the test and control encasements will be compared statistically if the data are appropriate.

SCHEDULE OF EVENTS:

<u>DATE</u>	<u>PROCEDURE</u>
Day 0	Test Conducted
End of Study	Telephone Report
Within following 30 days	Written Report
After the Written Report	Samples 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.

	
William Gaynor	Robert Sloane
Study Coordinator	Just Encase My Mattress Inc.
ICR, Inc.	
Date 12/4/09	Date 9/27/09



RAW DATA COLLECTION SHEET

Test Start Date:

Date of Last Blood Meal:

Time of exposure:

Test 'Mattress' or Control 'Mattress' (Circle one)

Replicate	Total # released	# recovered	
		inside	outside
1	Adults:		
	Nymphs:		
2	Adults:		
	Nymphs:		
3	Adults:		
	Nymphs:		
Total	Adults:		
	Nymphs:		
Mean	Adults:		
	Nymphs:		

Notes:

Recording Technician/Date:

Study Coordinator/Date:



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APPENDIX II: SPREADSHEETS



Data Reduction						
Project # 459-0			Sponsor: Just Encase My Mattress, Inc			Date: 10/20/09
Rep	Stage	Total #	# recovered inside	% recovered inside	avg % adults recovered insid	avg % nymph recovered insi
Control						
1	Adults	10	6	60		
	Nymphs	10	5	50		
2	Adults	10	3	30		
	Nymphs	10	3	30		
3	Adults	10	3	30		
	Nymphs	10	6	60	40	46.66666666666666
Ultraguard Mattress Protector						
1	Adults	10	0	0		
	Nymphs	10	0	0		
2	Adults	10	0	0		
	Nymphs	10	0	0		
3	Adults	10	0	0		
	Nymphs	10	0	0	0	0



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APPENDIX III: RAW DATA SHEETS



RAW DATA COLLECTION SHEET

Test Start Date: 10/6/09

Date of Last Blood Meal: 10/5/09

Time of exposure: 10/6/09

Observation Date 10/12/09

Test 'Mattress' or Control 'Mattress' (Circle one)

Replicate	Total # released	# recovered	
		inside	outside
1	Adults:	0	10
	Nymphs:	0	10
2	Adults:	0	10
	Nymphs:	0	10
3	Adults:	0	10
	Nymphs:	0	10
Total	Adults:	0	30
	Nymphs:	0	30
Mean	Adults:	0	10
	Nymphs:	0	10

Notes:

Recording Technician/Date: *mgs 10/12/09*

Study Coordinator/Date: *William J. Gaynor 10/12/09*



RAW DATA COLLECTION SHEET

Test Start Date: *10/6/09* Date of Last Blood Meal: *10/5/09*

Time of exposure: *10/6/09* Observation Date *10/12/09*

Test 'Mattress' or Control 'Mattress' (Circle one)

Replicate	Total # released	# recovered	
		inside	outside
1	Adults:	6	4
	Nymphs:	5	5
2	Adults:	3	7
	Nymphs:	3	7
3	Adults:	3	7
	Nymphs:	6	4
Total	Adults:	12	18
	Nymphs:	14	16
Mean	Adults:	4.0	6.0
	Nymphs:	4.7	5.3

Notes:

Recording Technician/Date: *MJA 10/12/09*
Study Coordinator/Date: *William J. Chapman 10/12/09*