No. 14M-RPTJUL016

July 15, 2014

To RAYCOP JAPAN INC.

Test Report

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1. Test name Consideration of vacuum cleaner's allergen removing performance

2. Purpose of test In this test, we will use a vacuum cleaner to suction a bedding that has been artificially contaminated with mite allergen (pseudo-contaminated bedding), and we will measure the amount of mite allergen found on the pseudo-contaminated bedding after suction to consider the vacuum cleaner's mite allergen removing performance.

3. Material and method

Vacuum cleaner Raycop RE-100
Raycop RS-300

Suction target Bedding that has been artificially contaminated with mite allergen
Mattress pad (cloth cover/100% cotton, filling material/100% polyester)
Mattress pad with a culture medium[*1] of Dermatophagoides pteronyssinus or house dust sprayed[*2] and spread over the surface covered with a sheet (65% polyester, 35% cotton)
[*1] Includes living mites, dead mites, excrement, and mite feed
[*2] We only tested RS-300 with house dust.

Measured allergen Dermatophagoides pteronyssinus culture medium:
Allergen derived from the excrement of Dermatophagoides pteronyssinus (Der p 1)
House dust [#19904, ITEA]:
Allergen derived from the excrement of Dermatophagoides farinae (Der f 1)

Testing method We sprayed a designated amount of Dermatophagoides pteronyssinus culture medium or house dust on the middle of a 10cm x 10cm mattress pad piece. Then, we covered a 10cm x 10cm sheet over the part that was sprayed, and we fixed the four corners of the sheet to the mattress pad piece with tape. We took this mattress pad piece and inserted it into a 10.3cm x 10.3cm mattress pad hole, and then we fixed it there with tape and called this a pseudo-contaminated bedding (Figure 1).
We used the target vacuum cleaner to suction the pseudo-contaminated bedding the designated number of times. Then, we removed the mattress pad piece from the pseudo-contaminated bedding after suctioning and separated it into the sheet (hereinafter "surface") and the mattress pad (hereinafter "inside"), and we used them as the test specimen. Next, we extracted the allergen from each test specimen and used the Sandwich ELISA method to measure either the Der p 1 or Der f 1 amount of mite allergen.

Test condition As shown in Table 1

Number of tests n=3

Extraction of allergen We added an extraction buffer to each of the test specimen, consisting of the surface and inside, and extracted allergen for two hours at room temperature. We then collected the extracted liquids and conducted centrifugal processing to each of them to obtain their supernatant fluid. We used this supernatant fluid as the measurement sample.

Data processing We indicated the results based on the mite allergen amount (ng) per test specimen. In addition, we used the formula shown below to calculate the removal rate under each suction condition.

Reduction rate (%) = (Y-X)/Y x 100

X: Amount of mite allergen on test specimen after suction (ng/piece)

Y: Amount of mite allergen on the object of comparison without suction (ng/piece)

Allergen measurement method Enzyme-linked immunosorbent assay (Sandwich ELISA)

Sandwich ELISA/We pre-coated solid-phase primary antibodies in each well of a 96-well microplate to capture the allergen. Next, we made secondary antibodies, which were labeled in advance, react to the enzyme and substrate in order. We measured the absorbance of each color-producing well to calculate the antigen level of the specimen from a standard curve.

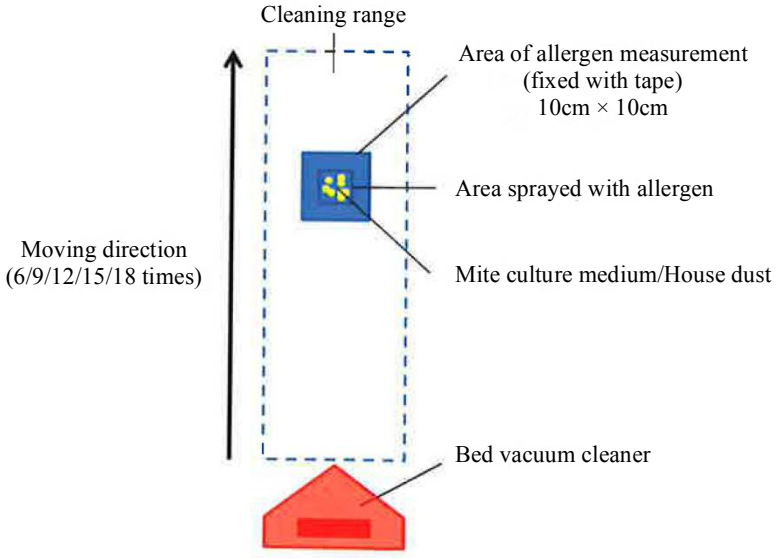

Figure 1. Schematic diagram of the experiment

Table 1. Test conditions (suction time, number of suctions, contamination amount)

Vacuum cleaner	Target allergen	Conditions	Suction time*1 (sec/m^2)	Number of suctions*2 (times)	Contamination amount*3 (mg/m^2)
RE-100	Der p 1 (Dermatophagoides pteronyssinus culture medium)	4 min/m^2	20	12	1857
		5 min/m^2	20	15	1857
		6 min/m^2	20	18	1857
		No suction	-	-	1857
	Der f 1 (House dust)	4 min/m^2	20	12	5900
		5 min/m^2	20	15	5900
		6 min/m^2	20	18	5900
		No suction	-	-	5900
RS-300	Der f 1 (House dust)	2 min/m^2	20	6	5900
		3 min/m^2	20	9	5900
		4 min/m^2	20	12	5900
		No suction	-	-	5900

*1 Suction time with vacuum cleaner per area of bedding conforms with the guidelines created by the Japanese Society of Allergology.

*2 The number of times that the vacuum cleaner completes a one-way distance (forward motion) of the pseudo-contaminated bedding
The number of suctions is determined based on the time spent (minutes) to suction 1m^2 surface area in the "conditions" and based on *1 described above.

*3 The amount of Dermatophagoides pteronyssinus or amount of house dust per 1m^2 of pseudo-contaminated bedding.

4. Results

*Removal rate (%) = [(No suction) - (Target)] / (No suction) x 100

Table 2. Test results of RE-100 (Sprayed object: Dermatophagoides pteronyssinus; Measurement target: Der p 1)

Conditions	n	Surface		Inside		Notes
		Der p 1 (ng/piece)	Removal rate* (%)	Der p 1 (ng/piece)	Removal rate* (%)	
4 min/m^2	1	188.5		2149.9		20sec/m^2 Number of suctions: 12 times
	2	218.1		3137.4		
	3	185.6		2354.3		
	average	**197.4**	**-88.16**	**2547.2**	**70.65**	
5 min/m^2	1	225.3		1899.0		20sec/m^2 Number of suctions: 15 times
	2	197.5		2120.5		
	3	191.6		1868.7		
	average	**204.8**	**-95.19**	**1962.7**	**77.39**	
6 min/m^2	1	151.7		1840.2		20sec/m^2 Number of suctions: 18 times
	2	175.4		1990.4		
	3	157.0		1638.3		
	average	**161.4**	**-53.82**	**1823.0**	**79.00**	
No suction	1	82.8		8705.1		
	2	76.6		8841.0		
	3	155.3		8492.0		
	average	**104.9**	**-**	**8679.4**	**-**	

Table 3. Test results of RE-100 (Sprayed object: house dust; Measurement target: Der f 1)

Conditions	n	Surface		Inside		Notes
		Der f 1 (ng/piece)	Removal rate* (%)	Der f 1 (ng/piece)	Removal rate* (%)	
4 min/m^2	1	68.6		972.9		20sec/m^2 Number of suctions: 12 times
	2	73.1		1338.2		
	3	67.6		1019.8		
	average	**69.8**	**0.45**	**1110.3**	**86.33**	
5 min/m^2	1	89.0		1743.3		20sec/m^2 Number of suctions: 15 times
	2	60.1		999.4		
	3	145.1		897.7		
	average	**98.0**	**-39.87**	**1213.4**	**85.06**	
6 min/m^2	1	80.7		1033.0		20sec/m^2 Number of suctions: 18 times
	2	62.8		963.2		
	3	70.1		763.4		
	average	**71.2**	**-1.58**	**919.9**	**88.68**	
No suction	1	37.9		7077.0		
	2	92.3		8805.1		
	3	80.1		8487.3		
	average	**70.1**	**-**	**8123.2**	**-**	

Table 4. Test results of RS-300 (Sprayed object: house dust; Measurement target: Der f 1)

Conditions (Suction time)	n	Surface		Inside		Notes
		Der f 1 (ng/piece)	Reduction rate* (%)	Der f 1 (ng/piece)	Reduction rate* (%)	
2 min/m^2	1	85.4		1097.6		20sec/m^2 Number of suctions: 6 times
	2	132.3		1516.0		
	3	90.0		1402.9		
	average	**102.6**	**70.69**	**1338.8**	**86.22**	
3 min/m^2	1	89.0		1305.1		20sec/m^2 Number of suctions: 9 times
	2	66.6		1266.7		
	3	75.0		1411.8		
	average	**76.9**	**78.04**	**1327.8**	**86.34**	
4 min/m^2	1	66.9		1122.5		20sec/m^2 Number of suctions: 12 times
	2	33.3		950.4		
	3	60.9		1146.0		
	average	**53.7**	**84.65**	**1073.0**	**88.96**	
No suction	1	510.4		9486.4		
	2	323.3		9768.8		
	3	216.3		9899.8		
	average	**350.0**	**-**	**9718.3**	**-**	

5. Summary

1) We suctioned a bedding that has been artificially contaminated with mite allergen (pseudo-contaminated bedding) for the designated number of times using vacuum cleaner “Raycop RE-100” or “Raycop RS-300.” We measured the amounts of mite allergen, Der p 1 or Der f 1, remaining on the pseudo-contaminated bedding after suctioning to consider the mite allergen contamination removal effects of vacuum cleaner “Raycop RE-100” or “Raycop RS-300.”

2) When spraying Dermatophagoides pteronyssinus culture medium (target mite allergen Der p 1), the removal rate on the inside of the bedding using vacuum cleaner “Raycop RE-100” in relation to the object of comparison, i.e. “no suction,” was 70.65% under the condition of “4 min/m^2,” 77.39% under the condition of “5 min/m^2,” and 79.00% under the condition of “6 min/m^2” (Table 2).

3) When spraying house dust (target mite allergen Der f 1), the removal rate on the inside of the bedding using vacuum cleaner “Raycop RE-100” in relation to the object of comparison, i.e. “no suction,” was 86.33% under the condition of “4 min/m^2,” 85.06% under the condition of “5 min/m^2,” and 88.68% under the condition of “6 min/m^2” (Table 3).

4) When spraying house dust (target mite allergen Der f 1), the removal rate on the inside of the bedding using vacuum cleaner “Raycop RS-300” in relation to the object of comparison, i.e. “no suction,” was 86.22% under the condition of “2 min/m^2,” 86.34% under the condition of “3 min/m^2,” and 88.96% under the condition of “4 min/m^2” (Table 4).

6. Supplementary information

This test considers the comparison between the specimen used in this test and the object of comparison. As such, the test results cannot be compared with other tests or experiments.

Test date: July 1 - July 9, 2014

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Appendix **1. Sandwich ELISA protocol**

Pre-coated solid-phase antibodies to allergen in each well of a microplate
↓
Post-coating after cleaning
↓
Add sample and allergen of known concentration (for creating standard curve) after cleaning
↓
Add labeled antibody to allergen after cleaning
↓
Add enzyme reagent after cleaning
↓
Add substrate after cleaning
↓
Measure after reaction stops

2. Total number of living mites in the Dermatophagoides pteronyssinus culture medium

No.	1	2	3	4	5	Average
Culture medium (mg)	24.7	29.7	24.8	25.1	24.6	25.78
Number of mites (mites)	93	110	110	119	92	104.8
Number of mites (mites/g)	3,765	3,704	4,435	4,741	3,740	4,077

We calculated the number of mites in accordance with JIS L 1920. We converted the actual measured value to the value per 1g of mite culture medium, and we considered the average value of n=5 to be the number of living mites per 1g of the said culture medium.

We calculated the ratio (average value) of the number of mites on the surface and inside of the mattress described in the reference below. Then, we carried out a provisional calculation of the number of mites on the entire bed based on the number of surface mites described in the reference. We designated the minimum value of the values obtained through this calculation as the amount of contamination under each test condition.

Reference: Midori Yoshikawa: The Ecology and Control of Mites of Houses (2), House and household insect pests 14(1), 13-25, 1992

3. Spraying amount of house dust

With regards to the spraying amount of Dermatophagoides pteronyssinus culture medium, we made sure that the total amount is 59mg/100cm^2 after mixing mite feed in relation to the weight of the contamination amount calculated above [(*1)].

The spraying amount of house dust is in accordance with this amount.

*1 This is in order to prevent variation in value caused by spraying a small amount