Immunological analysis on the effectiveness of vacuuming and carpet washing in the removal of common indoor allergens from carpet

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Abstract

Background: The most common allergies in Australia are house dust mites, cats, peanut and grass pollen, with allergen avoidance the most important aspect of allergy treatment. Carpets are a known reservoir of allergens and much of modern society spends most of their time indoors, and thus, the indoor environment is a key place to implement allergen avoidance by vacuuming and washing interventions. In particular, cat allergies, as represented by Fel d 1, are especially common in Australia, with allergen avoidance being paramount for allergy treatment. Effective removal of Fel d 1 due to its molecular size and characteristics is especially challenging and as it’s an effective bio marker of allergens being present as dander, hair and free allergen molecule, it is the best allergen candidate to assess removal. Thus, its removal by alternate cleaning methods is the focus of this study, as if any cleaning approach can effectively remove Fel d 1 then such approach will be similarly successful in removing all allergens.

Objectives: In this study, we aimed to assess the effectiveness of vacuuming and carpet washing in the removal of the major cat allergen, Fel d 1 by spiking different carpet samples with cat hair, cat dander and vacuum dust and performing several vacuuming and washing interventions on the different carpet types (wool, nylon and blend).

Methods: An allergen matrix was formed by spiking sand and collected vacuum dust with cat dander and hair. Carpet samples treated with the allergen matrix underwent a standardised vacuuming or washing intervention and the recovered samples were assessed for presence of Fel d 1 using a commercially available Fel d 1 ELISA. A number of market leading carpet washers (Bissell Revolution, Crosswave and SpotClean) and vacuums (Bagless Stick, Bagless Barrel and Bagged Barrel) were selected according to 'most sold' and 'best performance'.

Results: The Bissell Revolution removed more Fel d 1 from carpet than the CrossWave (diff = 40.28, p = <0.000001), SpotClean (diff = 79.16, p = <0.000001), Stick (diff = 94.86, p = <0.000001), Bagless Barrel (diff = 98.06, p = <0.000001) and Bagged Barrel (diff = 98.26, p = 000001) vacuum interventions. In addition, Revolution removed the most allergens from the nylon carpet, followed by the blend and then wool.

Conclusion: The Bissell Revolution performed the best at removing Fel d 1 from nylon, blend and wool carpet, amongst the other water-based cleaners tested. In addition, all water cleaners significantly outperformed traditional ‘dry’ vacuuming machines removing up to 66 times more allergens.
Keywords: Indoor allergens, cat allergens, Fel d 1, carpet washing, carpet washers, carpet vacuums, carpet vacuuming, allergen removal, allergen avoidance.
Introduction

Allergens exist in large numbers in our everyday surroundings, consisting of foreign proteins or glycoproteins from animals, plants and chemicals. Some individuals may possess an allergy which can arise from coming into contact with some foods including peanuts, dairy, soy, tree nuts, gluten, shellfish and eggs (Sicherer and Sampson, 2014), whilst others may have an allergy to chemicals such as latex and medicines such as penicillin and anaesthetics (Bhole et al., 2012; Burkhart et al., 2015; Macy, 2014). Common outdoor allergens arise from venom from insects such as bees and wasps, as well as mould, weed pollen, tree pollen and grass pollen. Outdoor allergens have the potential to penetrate indoor areas, however this is generally at a lower concentration compared to the outdoor environment (Burge and Rogers, 2000). Dust mites, pets and fungi are the main derivatives of indoor allergens. It is regarded that the majority of the people in the developed world spend the majority of their time indoors (Spengler and Sexton, 1983), which can allow for exposure to an indoor allergen to become chronic. Chronic exposure to common indoor allergens can lead to serious allergic respiratory diseases, such as asthma (Pomes et al., 2016), which not only burden the health system, but can also prove fatal.

Key findings in the report ‘Economic Impact of Allergies’ released by the Australian Society for Clinical Immunology and Allergy in 2007 state that 4.1 million (19.6%) of Australians have an allergy at a calculated cost of approximately $7.8 billion per annum. The report added that if this trend continues, there will be a 70% increase in the number of Australians affected from allergies by the year 2050. The report also found that allergies in children affect sleep, impair learning, memory and behaviours, and in adults lead to decreased quality of life, absenteeism from work and decreased productivity whilst at work which contribute to significant financial burden (Australian Society of Clinical Immunology and Allergy (ASCIA), 2007).

There are two phases of an immunological response to an allergy. The cell mediated or primary response (sensitization) is the first phase and refers to the initial exposure, which leads to the production of IgE in response to the foreign allergenic antigen. The second phase is known as the humoral or secondary response, which involves secondary exposure
to the same allergen and produces the excessive immune response (von Bubnoff et al., 2001).

An accurate diagnosis of an allergic disease and the suspect allergens allows for the correct treatment option to be determined. Skin prick testing is commonly performed for allergy diagnosis to determine a treatment (Canoncia and Ciprandi, 1989). However, allergen minimisation, or allergen avoidance, is the most effective form of treatment and involves consciously evading the known allergen that elicits an immune response (Australian Society of Clinical Immunology and Allergy (ASCIA), 2016). This method primarily treats food allergens, such as shell fish, and venom from insects, such as bees and wasps, which can be easy to implement as it only requires knowledge of precautions to be taken. On the other hand, it is difficult to avoid some allergens such as grass pollen, due to the nature of its unforeseen movements, and thus efforts to reduce allergen exposure should be focused. Cleaning of the indoor environment to remove allergenic proteins is necessary to decrease allergen exposure, however, there is evidence to suggest that vacuuming alone is not sufficient in reducing dust mite exposure (Cipriani et al., 2017).

Fel d 1 is one of the most important and common allergens in Australia (Australian Society of Clinical Immunology and Allergy (ASCIA), 2016). It is the major cat allergen, which is found on cat skin, fur and in salivary sebaceous glands. Cat allergen can adhere to surfaces and furs and generally settle on carpets, mattresses and curtains, where their presence can remain for a long time. It also has to ability to adhere to small dust particles (1-20um in size), allowing the allergen to be suspended in the air for extended lengths of time (Bush et al., 1998). This influences the allergen’s environmental distribution and contact with humans (Zahradnik and Raulf, 2014) as other allergens, such as HDM, settle rapidly from the air (de Blay et al., 1991). Thus, this makes Fel d 1 as the ideal allergen to assess its removal by dry vacuums and wet carpet washers since its successful removal would mean the removal of other allergens. Removal of pets from the household or keeping them outside is recommended to prevent allergen exposure, however families are generally reluctant to part with pets and it can take several weeks for allergen levels to decrease after pet removal (Wood et al., 1989). Tannic acid has been well studied in its ability to denature proteins, including Feld 1, and has been shown to reduce cat allergens by 80% on furniture and
carpets (Woodfolk et al., 1994). 0.05% sodium hypochlorite has also been shown to inactivate allergens, but frequent use can cause respiratory irritation (Portnoy et al., 2012; Zock et al., 2009). The use of dry heat is not recommended to reduce allergen exposure because Fel d 1 is very thermostable and would require treatment at 140°C for 60 minutes to partially remove allergens (Cain et al., 1998). Other studies have found that using of High Efficiency Particulate Arrestance (HEPA) air filters can decrease Fel d 1 allergen (Sublett et al., 2010).

A control study conducted by Popplewell et al., (2000) showed that vacuums with HEPA filters removed significant amounts of Fel d 1 allergen from bedroom carpet (median difference = 193ng/m² of carpet) and living room carpet (median difference = 185ng/m² of carpet) over a 12-month period resulting in improved clinical outcomes for allergy sufferers (Popplewell et al., 2000). The same study also concluded that regular vacuum cleaners remove insignificant amounts of both dog and cat allergens over a 12-month period (Popplewell et al., 2000). Studies providing evidence in relation to the amount of Fel d 1 or allergens generally removed by vacuuming versus washing carpets are few, hence, the overall aim of this study was to provide solid evidence in the effectiveness of Fel d 1 allergen removal by indoor cleaning techniques, such as vacuuming versus carpet washing.

Carpets can store and resuspend allergens and the dust recovered from carpet is helpful in qualifying and quantifying allergens that individuals are exposed to (Topp et al., 2003). It is therefore important to investigate carpets as a reservoir and source of indoor allergens (Adgate et al., 2013). It has been found that carpets with low-pile density and height, fluorocarbon coated fibres, square-hollow fibre shape and high denier per filament make it easier to remove HDM allergens (Causer et al., 2004; Lewis et al., 1998). These results have followed a common-sense approach, carpets that are easier to clean means more allergens are removed. However, studies comparing how effective vacuum cleaners and washers are at removing allergens from different carpet types do not exist, making this study to be the first of its kind to analyse these effects. The decision was made to include the most popular carpet types of wool, synthetic and blend (wool/synthetic) and exclude pile height and density for simplicity, as this is the first time this type of study has been conducted. The
popularity of carpet types was advised by Harvey Norman Australia, based upon their sales data and so this was used as an indicator of common household carpet types.

**Materials and Methods**

**Allergen Matrix**

An allergen matrix used to spike the carpet samples was based on the ‘test dust’ as described in the ATSM International Standard: *Evaluation of Carpet Embedded Dirt Removal Effectiveness of Household/Commercial Vacuum Cleaners (ASTM Int, 2017)*. The matrix consisted of 90% sand (Bunnings Warehouse, AUS) and 10% vacuum dust (collected by cleaners from Deakin University’s Burwood, Waurn Ponds and Waterfront campuses) and was stored in a bucket sterilised with 80% ethanol. Fel d 1 allergen material (cat dander and hair) were purchased and imported from Stallergenes Greer (USA) and 5 grams of each were added to the matrix. The matrix was then mixed with a sterilised Ozito 1050W Multi-Purpose Mixer (Bunnings Warehouse) and was analysed for Fel d 1 by a Fel d 1 sandwich ELISA kit (Indoor Biotechnologies), according to manufacturer’s instructions.

**Carpet and Vacuuming Selection**

54m² of popular carpets, each cut into 1x1m squares, were purchased from Harvey Norman (Waurn Ponds, AUS) that included 18m² each of wool, synthetic and wool-synthetic blend. The popularity of carpet types was advised by Harvey Norman Australia as determined by their sales data and this was used as being representative of common household carpet types. Three water-based cleaners, Bissell Revolution, Bissell CrossWave and Bissell SpotClean, were supplied by Bissell Australia for analysis. Three traditional vacuum cleaners were selected based on ‘most sold’ and ‘best performance’ as determined by Harvey Norman and included each of a ‘stick vacuum’, ‘bagless barrel’ and ‘bagged barrel’ type.

**Carpet and Vacuum Preparation and Spiking**

Batons of 1.2m long were cut to size and screwed to the outer edge of a 1.2 x 1.2m² plywood board to construct a platform on which the carpet samples can be placed and securely held for carpet preparation and treatment, see *Figure 1*. All carpet pre-conditioning and preparation was carried out on this board. A carpet sample was placed on the carpet
preparation board and pre-conditioned by running a rotating agitator vacuum cleaner (Vax Cordless Blade) for 5mins over the test area to remove carpet fluff. A 2.6 x 3.6m plastic drop sheet (Bunnings Warehouse) was placed on the carpet preparation board underneath the carpet sample and 100 grams of allergen matrix was evenly scattered onto the carpet by hand and scratched in with a sterile hand rake (Bunnings Warehouse) for 2.5mins. The carpet was then rolled with a paint roller (Bunnings Warehouse) for 2.5mins that was covered with a single-use plastic sleeve, the plastic sleeve was replaced after each rolling. The carpet was then wrapped with the plastic drop sheet, which was then taped closed, labelled and left overnight at room temperature prior to the vacuuming intervention. This process was repeated for all 54 pieces of 1 x 1m² carpet with the equipment being sterilised with 80% ethanol between spiking carpet samples to avoid any cross-contamination.

The Bissell SpotClean, stick vacuum, bagless barrel vacuum and bagged barrel vacuums were pre-conditioned by running them for 1 hour without bristles being engaged, as according to the ATSM vacuuming standard. Both the Bissell Revolution and CrossWave were run for 1 hour with bristles engaged that were not in contact with any surface. The Bissell SpotClean, bagged barrel, bagless barrel and bagged barrel vacuums suction and assembly were set in line with manufacturer’s specifications prior to intervention. All three Bissell washers were filled with water at 60ºC after each intervention, and specific settings included using the ‘Rug’ cleaning setting for the CrossWave, and ‘Deep Clean’ for the Revolution, according to manufacturer’s instructions.
Vacuuming Intervention Pattern and Dust Retrieval

The pattern for vacuuming was adopted by the ASTM International Standards for vacuum analysis (ASTM Int, 2017) and was determined by the number of vacuum nozzlewidths that could fit on one meter of the carpet. Each vacuum nozzle length was measured and divided into 1 meter, the value attained was the corresponding pattern used according to the standard. Figure 2 shows the cleaning pattern for Bissell Revolution, Bissell CrossWave, bagless barrel vacuum and bagged vacuum. Figure 3 shows the pattern for the stick vacuum and Figure 4 shows the cleaning pattern adopted for the Bissell SpotClean. The starting and ending point was always at the bottom right hand corner of the carpet, except for the Bissell SpotClean (Figure 4), with the instrument operator positioned against the lay of the carpet, see Figure 5. Each pattern consisted of 16 strokes, according to Annex 2 of the ASTM vacuum standard, with a stroke moving at the speed of 0.1m/s, therefore each intervention lasted approximately 160 seconds.

After intervention, the Bissell machines retrieved the allergen matrix in solution. The amount of solution was measured (mL), recorded and poured into plastic food containers with as much of the residual ‘mud’ removed from the machine as possible. The dust from the stick and bagless barrel vacuums was emptied according to the manufacturer’s instructions into a plastic bucket, this was then transferred into plastic food containers and weighed. With the bagged barrel vacuums, the bag was cut open and as much allergen matrix as possible was removed and placed in plastic food containers prior to weighing. All vacuum collection bins were washed out and sterilised with 80% ethanol between carpet interventions to avoid cross-contamination.
Figure 2: N = 4: cleaning pattern for Revolution, CrossWave and barrel vacuums

Figure 2: Shows the cleaning pattern adopted for the Bissell Revolution, Bissell CrossWave, bagless barrel vacuum and bagged barrel vacuum. The pattern used is determined by how many times the nozzle length of the vacuum can be divided into 1 meter (N), in this pattern N = 4. Note that the beginning and end points are in the same position.
**Figure 3: N = 5: stick vac cleaning pattern**

![Diagram of stick vac cleaning pattern with N = 5.]

*Figure 3: Shows the cleaning pattern intervention adopted for the stick vacuum. The pattern used is determined by how many times the nozzle length of the vacuum can be divided into 1 meter (N), in this pattern N = 5. Note that the beginning and end points are in the same position.*

**Figure 4: N = 8: SpotClean cleaning pattern**

![Diagram of SpotClean cleaning pattern with N = 8.]

*Figure 4: Shows the cleaning pattern intervention adopted for the Bissell SpotClean. The pattern used is determined by how many times the nozzle length of the vacuum can be divided into 1 meter (N), in this pattern N = 8. This pattern is not present in the ASTM vacuuming standard, to keep it comparable between patterns, the stroke number was kept to 16. Note that the beginning and end positions are not in the same position.*
Allergen Extraction

1 gram of dust from the stick, bagless barrel and bagged barrel vacuums was then placed in 20ml of PBS-T, pre-warmed to 60°C, in an individual 50ml sleeve tube. The reason why PBS-T was pre-warmed to 60°C was attributed to the fact that the Bissell washers utilised water heated to 60°C and thus, we used the same temperature to ensure temperature did not influence the amount of allergen extracted into solution. Indeed, separate tests demonstrated that heating had no influence on Fel d 1 removal. In addition, it should be noted here that only water was used in the Bissell products so had Bissell formulas been added its likely allergen removal by the Bissell cleaners would have increased further. This dust solution was then placed on a Ratek Platform Mixer on the speed setting 200 for 2 hours at room temperature and the resulting solution pipetted into 1mL centrifuge tubes and centrifuged at 1300 RPM for 5 minutes. The supernatant from the tubes were pooled into one 50mL tube, labelled and stored at -20°C prior to allergen analysis.

As the allergen matrix removed by the Bissell washers was already in solution, 10ml of this solution was placed into 10 x 1ml centrifuge tubes and centrifuged at 1300RPM for 5mins. The solution was mixed prior to aliquoting 1ml into centrifuge tubes to ensure an even allergen distribution. The supernatant was then pooled into a 50ml sleeve tube, labelled and stored at -20°C prior to allergen analysis. This process was repeated for all three of the Bissell washers.

Figure 5: Visualises the direction of the lay of the carpet in perspective to the person conducting the cleaning intervention. Figure 5a shows the position of the operator relative to ‘in the direction AGAINST the lay of the carpet’, whereas Figure 5b shows the position of the operator relative ‘in direction WITH the lay of the carpet’. The starting point of an intervention was always AGAINST the lay of the carpet, as shown in Figure 5a.
Allergen Quantification
The presence of cat allergen was quantified by Fel d 1 sandwich ELISA purchased and imported from Indoor Biotechnologies, India. Measurements were conducted in triplicate and each sample analysis was conducted at least 3 times.

Statistical Analysis
Graphical representation and statistical analysis of the Fel d 1 ELISA analysis of the allergen matrix and vacuuming intervention results were performed with Microsoft Excel and R Commander. A two-way ANOVA was utilised to determine if there was any significant difference in mean variation between groups, followed by a Tukey’s test to analyse for significant differences in between groups (vacuum and carpet type).
Results

ELISA and Statistical Analysis

Graphical representation and statistical analysis of the Fel d 1 ELISA analysis of the vacuuming interventions was performed with Microsoft Excel and R Commander.

Vacuum Intervention Analysis

The analysis of Fel d 1 extracted from each of the vacuums for all three of the carpet types was conducted independently at least 3 times, and a representative was chosen for statistical analysis. Table 1 summarises, and Figures 6-8 visualise, the average amount of Fel d 1 recovered by the vacuuming interventions from the three carpet types and Figure 9 allows for the visualisation of the standard error over each vacuum and carpet type.

Table 1: Summaries of the mean value for recovered Fel d 1 (ug), equivalent to 1g of allergen matrix, by the vacuuming interventions (Revolution, CrossWave, SpotClean, stick vacuum, bagless barrel vacuum and bagged barrel vacuum) from the blend, nylon and wool carpets. The values in round brackets represent percent recovery from the initial allergen matrix input of 2840ug of Fel d 1 per gram of allergen matrix. Highest allergen removal of 2725.80ug was by Revolution and lowest removal of 41.3ug was by bagged barrel, making wet cleaners up to 66 more effective than dry vacuums in allergen removal.

<table>
<thead>
<tr>
<th></th>
<th>Fel d 1 (ug) Recovered from Blend Carpet</th>
<th>Fel d 1 (ug) Recovered from Nylon Carpet</th>
<th>Fel d 1 (ug) Recovered from Wool Carpet</th>
<th>Average over 3 Carpet Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revolution</td>
<td>2284.82 (80.5%)</td>
<td>2725.80 (96%)</td>
<td>1766.75 (62.2%)</td>
<td>2259.12 (79.5%)</td>
</tr>
<tr>
<td>CrossWave</td>
<td>1343.27 (47.3%)</td>
<td>1818.82 (64%)</td>
<td>947.73 (33.4%)</td>
<td>1369.94 (48.2%)</td>
</tr>
<tr>
<td>SpotClean</td>
<td>378.66 (13.3%)</td>
<td>694.39 (24.5%)</td>
<td>361.89 (12.7%)</td>
<td>478.31 (16.8%)</td>
</tr>
<tr>
<td>Stick</td>
<td>92.72 (3.3%)</td>
<td>213.49 (7.5%)</td>
<td>222.61 (7.8%)</td>
<td>176.27 (6.2%)</td>
</tr>
<tr>
<td>Bagless Barrel</td>
<td>75.11 (2.6%)</td>
<td>260.18 (9.2%)</td>
<td>247.63 (8.7%)</td>
<td>194.30 (6.8%)</td>
</tr>
<tr>
<td>Bagged Barrel</td>
<td>41.30 (1.5%)</td>
<td>67.99 (2.4%)</td>
<td>69.92 (2.5%)</td>
<td>59.74 (2.1%)</td>
</tr>
</tbody>
</table>
Figure 6: Shows the mean Fel d 1 allergen recovered, from equivalent 1g of the allergen matrix, by the Bissell Revolution (Rev), Bissell CrossWave (CW), Bissell SpotClean (SC), Stick vacuum (Stick), Bagless Barrel vacuum (Bagless Barrel) and Bagged Barrel Vacuum (bagged Barrel) cleaning interventions from Blend carpet with standard error bars present.

Figure 7: Shows the mean Fel d 1 allergen recovered, from equivalent 1g of the allergen matrix, by the Bissell Revolution (Rev), Bissell CrossWave (CW), Bissell SpotClean (SC), Stick vacuum (Stick), Bagless Barrel vacuum (Bagless Barrel) and Bagged Barrel Vacuum (bagged Barrel) cleaning interventions from Nylon carpet with standard error bars present.
Figure 8: Shows the mean Fel d 1 allergen recovered, from equivalent 1g of the allergen matrix, by the Bissell Revolution (Rev), Bissell CrossWave (CW), Bissell SpotClean (SC), Stick vacuum (Stick), Bagless Barrel vacuum (Bagless Barrel) and Bagged Barrel Vacuum (bagged Barrel) cleaning interventions from Wool carpet with standard error bars present.

Figure 9: Shows the percentage of Fel d 1 recovered, from equivalent 1g of the allergen matrix, by the Bissell CrossWave, Bissell SpotClean, stick vacuum (Stick), Bagless Barrel vacuum (Bagless Barrel) and Bagged Barrel Vacuum (bagged Barrel) cleaning interventions from Blend, Nylon and Wool carpet with standard error bar present.
A two-way ANOVA was fitted to the percentage of Fel d 1 recovered by vacuums compared to the Revolution and found a statistically significant difference between the vacuum type and percentage of Fel d 1 removed compared to the Revolution (F = 215.29, p = < 2e-16). There was no statistically significant difference between the carpet type (F = 1.39, p = 0.263) and percentage of Fel d 1 removal compared to the Revolution, and any interaction effect between the vacuum and carpet type (F = 0.46, p = 0.906) was also found not to be statistically significant.

A Tukey’s test was applied to the two-way ANOVA. This found that there was a significant difference between the percentage of Fel d 1 removed by the Revolution compared to the CrossWave (diff = 40.28, p = <0.000001), SpotClean (diff = 79.16, p = <0.000001), Stick (diff = 94.86, p = <0.000001), Bagless Barrel (diff = 98.06, p = <0.000001) and Bagged Barrel (diff = 98.26, p = 0.000001) vacuums. There was no significant difference between Nylon and Blend carpet (diff = 4.18, p = 0.29), Wool and Blend (diff = 0.51, p = 0.98) and Wool and Nylon (diff = 3.67, p = 0.38) carpet types and the amount of Fel d 1 removed by vacuums compared to the Bissell Revolution.
**Discussion**

**Cleaner Intervention Analysis**

From this study our hypothesis that cleaning interventions will remove allergen has been proven correct. Detection of Fel d 1 in sandwich ELISA analysis of cleaner extracts has confirmed this, as seen in Table 1 and Figure 6-8. It was expected prior to the study that water-based carpet cleaning interventions would remove more allergen than traditional vacuums. As seen in Table 1, the Bissell Revolution has clearly recovered more Fel d 1 allergen (with an average of 79.5% of recovery from the initial allergen input) from all three carpet types compared to other interventions. From looking at Figures 6-8 a pattern can be seen with the Bissell Revolution removing the most allergen (up to 96% from the nylon carpet) followed by the Bissell CrossWave, Bissell SpotClean, stick vacuum, Bagless Barrel vacuum and the Bagged Barrel. This is similar over all carpet types with the only exception being that the Bagless Barrel recovered more allergen than the Stick vacuum on Blend carpet. Again, this trend shows that on average 77% more Fel d 1 allergen is removed by the water-based cleaners than traditional ‘dry’ vacuums.

Since Bissell Revolution was the best performer, with up to 96% Fel d 1 extraction from the nylon carpet, it was used as the benchmark intervention to compare the rest of the interventions to. Therefore, Figure 9 allows the visualisation of the percentage of Fel d 1 removed by the cleaner types compared to the Revolution on the different carpet types. It shows that the amount of Fel d 1 removed by the Revolution was statistically significant when compared to the other cleaners, as the standard error bars do not overlap. It also shows that carpet type has no impact on the ability of the cleaner at removing the allergen, in other words, the cleaners perform with similar trends on each carpet type. This is because the standard error bars for the carpet types within each vacuum type are overlapping. Both results are backed by the ANOVA results, which have confirmed that there is a statistically significant difference between the cleaner type and percentage of Fel d 1 removed when compared to the Revolution ($F = 215.29$, $p < 2e-16$), and no statistically significant difference between the carpet type ($F = 1.39$, $p = 0.263$) and percentage of Fel d 1 removal when compared to the Revolution. It also showed that there was no interaction
affect between carpet and cleaner type, again leading us to believe that the cleaner type is the most important factor in allergen removal.

The Tukey’s test conducted allows us to see the performance of each cleaner on each carpet type compared to Revolution. The results confirm that the Revolution removes more Fel d 1 from not just carpet in general, but all three of blend, nylon and wool. It also confirms that carpet does not influence the ability of cleaners at removing Fel d 1 allergen (Nylon and Blend carpet (diff = 4.18, p = 0.29), Wool and Blend (diff = 0.51, p = 0.98) and Wool and Nylon (diff = 3.67, p = 0.38)). It also tells us that the Revolution outperforms each individual cleaner type (CrossWave (diff = 40.28, p = <0.000001), SpotClean (diff = 79.16, p = <0.000001), Stick (diff = 94.86, p = <0.000001), Bagless Barrel (diff = 98.06, p = <0.000001) and Bagged Barrel (diff = 98.26, p = 000001) vacuums), on each carpet type.

As the Revolution is designed specifically for carpet cleaning, this was not a surprise to find that it out performed other appliances, but it was surprising how much more allergen was recovered compared to other cleaners. The CrossWave washer is primarily designed for cleaning of hard surfaces, such as hardwood, tiles etc, and not the fibre piles present in carpet. Hence, it was a surprise to find it removed up to ??% more allergen from carpets than traditional vacuums. The SpotClean is designed for cleaning small areas and not large carpeted areas, but also was superior to traditional vacuums in removing Fel d 1 despite this instrument not having a rotating brush feature like the other instruments.

the Stick, Bagless Barrel and Bagged Barrel vacuums offered significantly inferior ability to remove Fel d 1 from carpet when compared to the water-based cleaners. We believe comparing the difference in allergen removal by the Stick and Bagless Barrel vacuums is a fair comparison because manufacturer’s instructions were followed in assembly and operation of the appliances, and because the patterns were adopted by the ASTM vacuuming standard. There is always the possibility that minimal residual allergens were present inside the bins of the Stick and Bagless Barrel vacuums, particularly in the HEPA filters present. Analysis of the HEPA filters would need to occur to prove this, however, it could be argued that the addition of cat dander and hair, to represent the Fel d 1 allergen, are too large to become trapped in a HEPA filter, and theoretically, will be present in the
vacuum bin. Furthermore, the Bissell washers utilised water heated to 60°C and to ensure temperature did not influence the amount of allergen extracted into solution, the allergen extractions were carried out in PBS-T heated to 60°C for the Stick and Barrel vacuums. On the other hand, it may be argued that the comparison between the Bagged Barrel vacuum and the other vacuums is not a fair comparison. This is because the Bagged Barrel vacuum contents was collected in a bag designed in such a way that no dust, and therefore allergens, can escape the bag, fully containing the dust. Due to this design, it was very difficult, and almost impossible, to remove all the bags contents for ELISA analysis. Thus, it may be argued that this study does not fully represent the ability of the Bagged Barrel vacuum to remove allergen from carpet as there was still dust present in the collection bag. A possible solution to this in future applications would be to either replace the vacuum bag with a plastic bag or shred the collection bag and place it into PBS-T solution to extract the allergens prior to analysis.

It is thought that the amount of Fel d 1 detected was high in the water-based cleaners due to the allergen being dragged into solution immediately after being recovered, whereas in the ‘dry’ vacuums it may have become displaced. As the water-based machines removed significantly more Fel d 1 from carpet than traditional vacuums, this has led to the conclusion that water-based cleaners remove more Fel d 1 (and other allergens considering the heightened difficulty of removing Fel d 1) from carpet than traditional dry vacuuming machines. Therefore, individuals allergic to indoor allergens will likely have more success in removing indoor allergens with water-based cleaners than the traditional dry vacuums. In addition, disposal of dry vacuum dust from bagless vacuums presents another risk for potential allergen exposure post vacuum. On the other hand, disposal of waste from water-based cleaners may be a lot safer for the allergic individual as allergens are trapped in the water reservoir, which can be safely and easily disposed of, thereby minimising allergen exposure post allergen removal.

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