

Effectiveness of vacuuming and carpet washing in the removal of the major cat allergen, Fel d 1

To the Editor:

Carpets are a known reservoir of allergens and much of modern society spends most of their time indoors,¹ and thus, the indoor environment is a key place to implement allergen avoidance by vacuuming and washing interventions. Dust mites, pets and fungi are common sources of indoor allergens.² Chronic exposure to common indoor allergens can lead to serious allergic respiratory diseases, such as asthma,³ which not only burden the health system, but can also prove fatal. The aim of this study was to assess the effectiveness of vacuuming and carpet washing in the removal of the major cat allergen, Fel d 1.

Fel d 1 is one of the most important and common allergens in Australia.⁴ 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 the capacity to adhere to small dust particles (1–20 µm in size), allowing the allergen to be suspended in the air for extended lengths of time.⁵ This influences the allergen's environmental distribution and contact with humans⁶ as other allergens, such as house dust mite (HDM), settle rapidly from the air⁷ due to their association with larger particles.⁸ Thus, we decided to focus on Fel d 1 as a representative indoor allergen to assess its removal by dry vacuums and wet carpet washers.

From this study, our hypothesis that cleaning interventions can successfully remove Fel d 1 has been proven correct. Detection of Fel d 1 by a highly sensitive sandwich ELISA analysis of cleaner

extracts has confirmed this (Table 1 and Figure 1). Highest allergen removal of 2725.80 µg was by Revolution and lowest removal of 41.3 µg was by Bagged Barrel, making wet cleaners up to 66 more effective than dry vacuums in allergen removal. 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 with other interventions. A pattern can be seen with the Revolution removing the most allergen (up to 96% from the nylon carpet (Table 1)) followed by the Bissell CrossWave, Bissell SpotClean, Stick vacuum, Bagless Barrel vacuum and the Bagged Barrel (Table 1). This is similar over all carpet types with the only exception being that the Bagless Barrel recovered more allergen than the Stick on blend carpet, which was not statistically significant. 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 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 1 allows the visualisation of the percentage of Fel d 1 removed by the cleaner types compared with Revolution on the different carpet types. The amount of Fel d 1 removed by Revolution was statistically significant when compared to the other cleaners. 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. Both results are backed by the ANOVA results, which have confirmed

TABLE 1 Mean value (±SD) for recovered Fel d 1 (µg), equivalent to 1 g 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

	Fel d 1 (µg) Recovered from Blend Carpet	Fel d 1 (µg) Recovered from Nylon Carpet	Fel d 1 (µg) Recovered from Wool Carpet	Average Fel d 1 (µg) Recovered from All Samples
Revolution	2284.82 ± 106.69 (80.5%)	2725.80 ± 389.89 (96%)	1766.75 ± 99.85 (62.2%)	2259.12 (79.5%)
CrossWave	1343.27 ± 130.36 (47.3%)	1818.82 ± 62.05 (64%)	947.73 ± 155.64 (33.4%)	1369.94 (48.2%)
SpotClean	378.66 ± 48.64 (13.3%)	694.39 ± 53.10 (24.5%)	361.89 ± 79.26 (12.7%)	478.31 (16.8%)
Stick	92.72 ± 1.03 (3.3%)	213.49 ± 6.33 (7.5%)	222.61 ± 3.50 (7.8%)	176.27 (6.2%)
Bagless Barrel	75.11 ± 5.02 (2.6%)	260.18 ± 13.88 (9.2%)	247.63 ± 19.47 (8.7%)	194.30 (6.8%)
Bagged Barrel	41.30 ± 1.27 (1.5%)	67.99 ± 0.68 (2.4%)	69.92 ± 24.32 (2.5%)	59.74 (2.1%)

Note: The values in round brackets represent per cent recovery from the initial allergen matrix input of 2840 µg of Fel d 1 per gram of allergen matrix.

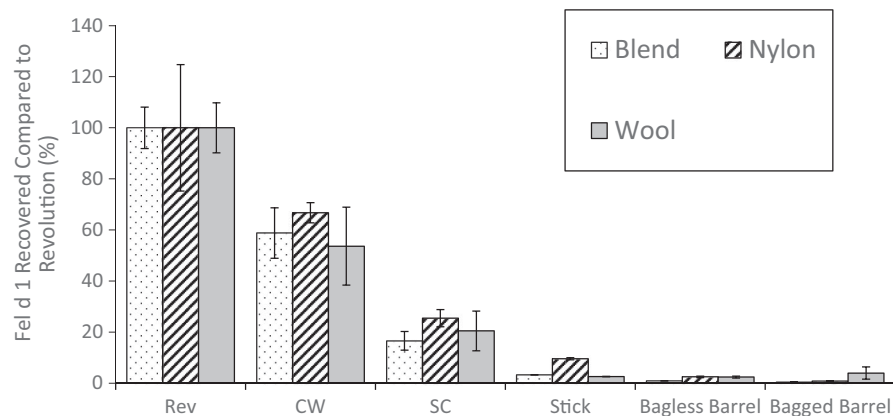


FIGURE 1 Fel d 1 allergen removal from different carpet types by different cleaning interventions. The percentage of Fel d 1 recovered by different cleaning interventions, when benchmarked to Bissell Revolution, is shown. The results represent the mean of three replicates within an experiment, and the error bars show standard deviation

that there is a statistically significant difference between the cleaner type and percentage of Fel d 1 removed when compared to Revolution ($F = 215.292$, $P = <2e-16^{***}$), and no statistically significant difference between the carpet type and percentage of Fel d 1 removal when compared to Revolution ($F = 1.388$, $P = .263$). It also showed that there was no interaction affect between carpet and cleaner type ($F = 0.457$, $P = .906$), again leading us to believe that the cleaner type is the most important factor in allergen removal.

The Tukey test conducted allows us to see the performance of each cleaner on each carpet type compared to Revolution (Figure 1). The results confirm that 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, wool and blend and wool and nylon). It also tells us that Revolution outperforms each individual cleaner type on each carpet type.

As Revolution is designed specifically for carpet cleaning, this was not a surprise to find that it outperformed other appliances, but it was surprising how much more allergen was recovered compared with other cleaners, which was up to 66 times more allergen from carpets than traditional vacuums.

The “dry” 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 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 standard⁹ across all cleaners (Figures S1-S5). However, a limitation of this study is that our results may not be generalised to older carpets until further experiments are performed.

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 airborne during the dust collection prior to allergen extraction. Although not tested in this study, water-based cleaners might also be efficient in removing other allergens from carpet, given the heightened difficulty of removing Fel d 1 from

home environments. 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 after 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 after allergen removal. In future studies, it will be important to consider other indoor allergens, other types of cleaning devices, such as robot vacuums, and other flooring and upholstery materials, as well as exposure levels to airborne allergens during vacuuming.

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CONFLICT OF INTEREST

The authors have no conflict of interest. Furthermore, there is no professional relationship between the authors of the work and Bissell Australia.

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Timothy B. Poole
Samuel Peter King
Cenk Suphioglu 

NeuroAllergy Research Laboratory (NARL), School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Waurn Ponds, Vic, Australia

Correspondence

Cenk Suphioglu, NeuroAllergy Research Laboratory (NARL),
School of Life and Environmental Sciences (LES), Faculty of
Science, Engineering and Built Environment (SEBE), Deakin
University, Geelong Waurin Ponds Campus, 75 Pigdons Road,
Geelong, VIC 3216, Australia.
Email: cenk.suphioglu@deakin.edu.au

ORCID

Cenk Suphioglu  <https://orcid.org/0000-0003-0101-0668>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Effectiveness of vacuuming and carpet washing in the removal of the major cat allergen, Fel d 1.

Online Supplement

Methods

Allergen Matrix

An allergen matrix used to spike the carpet samples was based on the 'test dust' as described in the American Society for Testing and Materials (ASTM) International Standard: *Evaluation of Carpet Embedded Dirt Removal Effectiveness of Household/Commercial Vacuum Cleaners*⁹. The matrix consisted of 90% sand (Bunnings Warehouse, AUS) and 10% vacuum dust (collected by cleaners from Deakin University's Burwood, Waurin 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 (Waurin 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 S1. 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 standard⁹. 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, 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 60°C 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 analysis⁹ and was determined by the number of vacuum nozzle widths that could fit on one

meter of the carpet. Each vacuum's nozzle length was measured and divided into 1 meter, the value attained was the corresponding pattern used according to the standard. Figure S2 shows the cleaning pattern for Bissell Revolution, Bissell CrossWave, Bagless Barrel vacuum and Bagged vacuum. Figure S3 shows the pattern for the Stick vacuum and Figure S4 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 S4), with the machine operator positioned against the lay of the carpet, see Figure S5, to establish direction of the lay of the carpet. 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, as the total time for vacuuming was 160 seconds and each stroke measured 1m.

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. It is important to note here that all of the dry vacuum canisters, filters and bags were brushed and collected for any residual dust between different treatments and cleaned between runs. Furthermore, to avoid any accidental loss of the cat allergens, this study used cat hair and dander as Fel d 1 allergen is attached on these substrates. Therefore, Fel d 1 would be more residual in the vacuum dust matrix for its successful removal by the different vacuum types.

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 (data not shown).

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 1300 RPM 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.

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. In detail, all Fel d 1 calculations focussed on the total amount of Fel d 1 extracted by each replicate for each vacuum intervention type. For wet vacuums, the total volume of the recovered liquid was measured and recorded. Similarly, for the dry vacuums, the total dust weight recovered was measured and recorded. In order to not overwhelm the Fel d 1 ELISA, dilutions of the wet vacuum liquid and dry vacuum dust suspension (initially 1g mixed with 20mL) were carried out and again recorded. Once determined in the Fel d 1 ELISA, against a Fel d 1 standard curve, the total amount of Fel d 1 recovered by each vacuum type was calculated, considering the different dilution rates, as well as total masses and volumes recovered by the dry and wet vacuums, respectively. Seeing that we have also performed Fel d 1 ELISA on 1g of the 100g allergen matrix that was used in the vacuum intervention experiments, we can then express the values in Table I as equivalent to 1g of the allergen matrix (i.e. we know how much total allergen went onto the carpet and how much total allergen was recovered by each vacuum type).

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).

Figure legends

Figure S1. Carpet anchoring during the intervention stage. Carpet sample on top of the plastic sheet to contain the carpet overnight, on the plywood board with the batons attached to the outer edge on the bottom.

Figure S2. Carpet cleaning pattern during the intervention stage. The pattern used for the Bissell Revolution, Bissell CrossWave, Bagless Barrel vacuum and Bagged Barrel vacuum is determined by how many times the nozzle length of the vacuum can be divided into 1 meter (N), in this pattern N = 4.

Figure S3. Carpet cleaning pattern during the intervention stage. The pattern used is for the Stick vacuum determined by how many times the nozzle length of the vacuum can be divided into 1 meter (N), in this pattern N = 5.

Figure S4. Carpet cleaning pattern during the intervention stage. The pattern used for the Bissell SpotClean 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.

Figure S5. Carpet lay direction during the intervention stage. (i) shows the position of the operator relative to 'in the direction AGAINST the lay of the carpet', whereas (ii) 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 (i).

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163 **Figures**

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165 Figure S1

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Figure S2

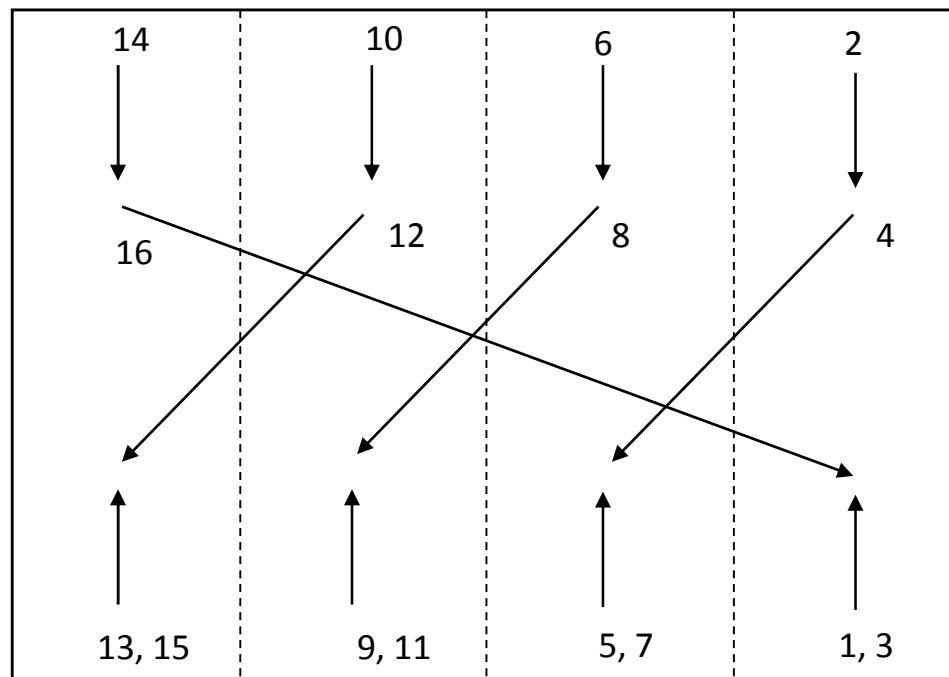


Figure S3

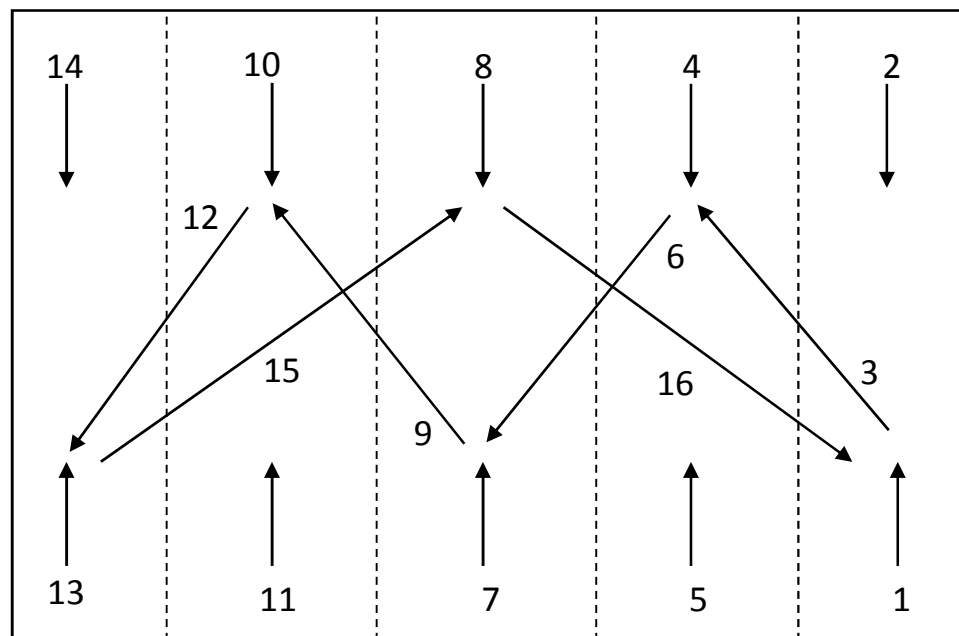


Figure S4

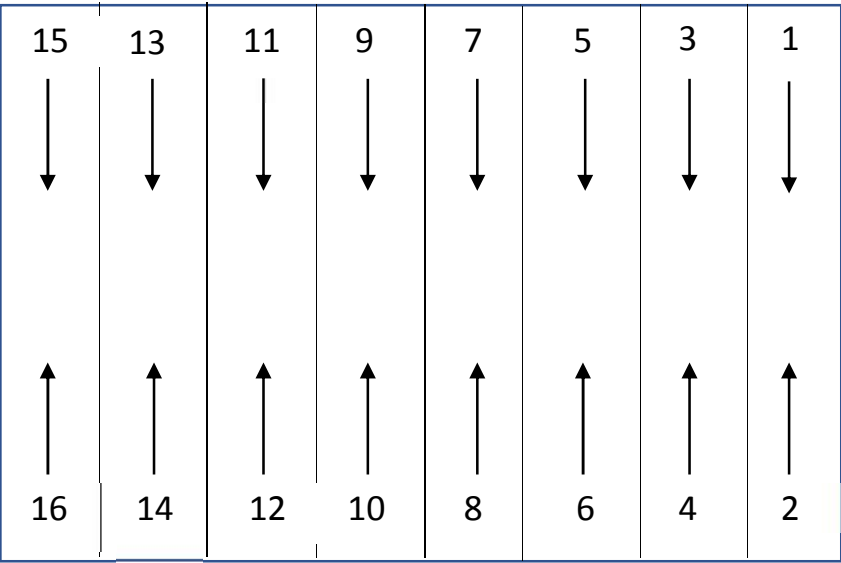


Figure S5

