Report on the effectiveness of the Airfree air steriliser manufactured under license of US Patent 5874050 at reducing the levels of Der p 1 (a major house dust mite allergen) on allergen placed within it for varying lengths of time.

Phase 1

Report No. Air/Mit/All/1

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This report consists of 7 numbered pages of which this is the first.
Disclaimer

The results described in this report were generated *in vitro*. The samples tested were accepted in good faith that they were representative of the intended final formulation(s)/product(s) and the test methods employed were used on the understanding that they were the most appropriate available at the time the tests were agreed. As such the results should be taken only as an indication of the potential for activity of the formulations or products under test. These results cannot be considered as confirmation that a formulation or product will work in a clinical or field application. Evidence for such activity can only be obtained from properly constructed and executed clinical or field trials.

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Aim

The aim of these experiments was to assess the effectiveness of the Airfree device at reducing the levels of Der p 1 (A major house dust mite allergen) in allergen placed within it for varying lengths of time.

Materials and methods

House dust mite allergen, derived from colonies of the house dust mite *Dermatophagoides pteronyssinus*, maintained by Insect Research and Development Ltd was placed into the centre of the incinerator for 1, 5 and 300 seconds using a probe. All of the experiments were conducted in a controlled climate chamber set to 25°C and 75% RH. The probe was constructed from straight metal wire measuring 120 mm with a diameter of 0.75 mm, a 5mm by 10 mm strip of autoclave tape was wrapped around the wire leaving an exposed surface of 2mm by 10mm. Approximately 0.001 grams of frozen culture containing high levels of allergen was placed onto this surface, calculations in the results section will take this variation into account. The incinerator was placed in the upright position and turned on for 24 hrs prior to the addition of the probes. The allergen was placed into the centre of the incinerator, through a small hole made in the grill at the bottom for 1,5 and 300 seconds (see figure 1), 3 replicates were conducted at each time interval and 3 control replicates were carried out at each time interval with the Airfree device switched off so the incinerator was at room temperature (25°C). The incinerator was 13 cm long so the tip of the probe was placed 7cm within it, in the central hole. The centre of the autoclave tap was inline with the centre of the incinerator.
After being placed in the incinerator the autoclave tape was gently removed from the wire and placed into a 25ml water tight container containing 5ml of dust extraction buffer (0.125M ammonium hydrogen carbonate buffer + 0.1% sodium azide). The container containing the allergen and dust extraction buffer was spun with a blood rotator for 1 hour. 1ml of liquid from each arena was then transferred to labelled Eppendorf tubes using a micro-pipette, and centrifuged at 6000rpm for 5 minutes. The supernatant liquid (0.2 ml) was removed from each Eppendorf tube and transferred to a new, labelled Eppendorf tube, after this the tubes were frozen until they were analysed for Der p 1.
Diagram showing the probe inserted into the incinerator

Fig 1: Diagram showing probe being inserted into the incinerator.
**Results (corrected)**

<table>
<thead>
<tr>
<th>Exp/Control</th>
<th>Mean ng Der p 1</th>
<th>% Reduction</th>
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</thead>
<tbody>
<tr>
<td>5 min exposure</td>
<td>6.82</td>
<td>97.95</td>
</tr>
<tr>
<td>5 min control</td>
<td>332.47</td>
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<td>169.66</td>
<td>70.60</td>
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<tr>
<td>1 second control</td>
<td>577</td>
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</table>

Table 1: Results

**Results and discussion**

The results indicate that the Airfree device has the potential to control the airborne house dust mite allergen Der p 1 extremely well. After just one second, the time which air/airborne allergens are thought to remain in the incinerator, the Airfree device was able to reduce the amount of the house dust mite allergen Der p 1 by an average (mean) of 70.6%. In the field the airborne allergen may be denatured even more as quite a lot of allergen was placed on the probe, some of the allergen on the probe may therefore have shielded the allergen beneath it from the heat. It was interesting to see that the longer the control samples were placed within the incubator the less allergen was detected on them, as the incubator was switched off it is likely that this reduction was caused by some of the allergen being rubbed off the probe. Longer exposure periods with the incinerator switched on resulted in higher % reductions in allergen concentrations when compared to the control, this indicates that repeated exposure to the
incinerator would reduce the amount of allergen further, although more tests would need to be conducted to confirm this.