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1. Purpose

Dust Free® seeks to have undertaken an assessment of the impact of its Air Knight® and Air Knight® IP3 Air Purification Systems on bioaerosol inactivation in a modified ASHRAE 52.2 style test duct connected to an environmental test chamber. This is an initial proposal for discussion and can be modified as required by Dust Free®.

airmid healthgroup (AHG) has also included allergens as another option that can potentially be assessed with the air purification system in the duct. This is subject to the clients requirements.

2. Proposal

This proposal is divided into 3 sections. Section (a) describes the micro-organisms and allergens that AHG recommends to be used for the assessment of the Air Knight® air purification systems. Sections (b) and (c) outline the proposed testing protocols.

(a) Bioaerosols and Allergens

Bioaerosols: The species of virus, bacteria and fungi chosen for this study are intended to act as indicator organisms demonstrating how other species from these classes of microorganisms are likely to behave.

AHG suggests a bioaerosol test panel consisting of a single fungal species (*Aspergillus niger*), a single bacterial species (*Staphylococcus epidermidis*) and a single virus species (MS2 Coliphage). Refer to Appendix 1 for detailed descriptions of each of these microorganisms. Please note that *A.niger* is introduced into the duct as dry spores in an inert dust, while the bacteria and virus will be nebulised in a liquid form into the duct.

Additional micro-organisms can be included as required such as *Mycobacterium smegmatis* as an additional bacterial species, *Cladosporium* or *Penicillium* species as additional fungi and Influenza A as an additional virus species. Other species can also be included at the suggestion of Dust Free®.

Allergens: AHG suggests using the house dust mite *Dermatophagoides pteronyssinus* allergen Der p1 and the domestic cat allergen Fel d1. Allergen test dust (ATD), which is composed of house dust mite allergen, cat allergen and horse dust will be used to introduce the allergens to the test duct. The particle size and distribution of ATD is comparable to dust found in homes and thus represents a reasonable and appropriate challenge for consumer products related to the home.

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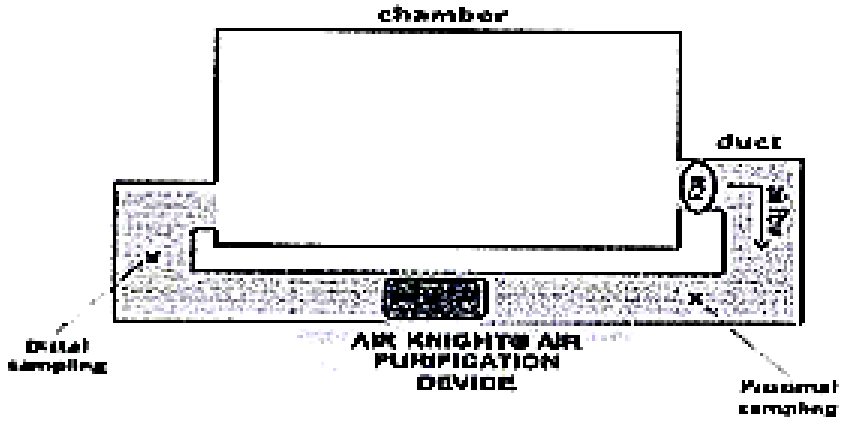
(b) Proximal and Distal Sampling in-duct ("Single Pass")

If Dust Free® wishes to consider testing their air purification system with a 'single pass' method, this is outlined in this section. The Air Knight® air purification system will be installed in the test duct as indicated in the diagram below. Photographs will be taken of the air purification system installation and e-mailed to Dust Free® to confirm that the set-up is correct.

The best approximation of a "single pass" would be achieved by running the duct fan and setting the chamber to a "full dump" of air. There may be some re-circulation of the air from the chamber back into the duct, but this would be negligible. At the distal end of the duct, a MERV 10 filter and 3M Filtrete 2400 MPR filter have been installed to prevent passage of airborne micro-organisms into the chamber, thus preventing contaminated air from being recirculated though the duct during testing.

The test duct will be decontaminated between test runs and then HEPA-filtered conditioned air will allowed to circulate in the duct from the adjacent environmental test chamber. The chamber will be then switched to full air dump for 5min to remove any traces of contaminated air prior to commencement of the next run.



The following test-run will be performed with the Air Knight® air purification device turned off, in triplicate i.e. 3 control runs, and then with the Air Knight® air purification device turned on, in triplicate i.e. 3 test runs (see Table 1 in Appendix 2 for further details):

- Bioaerosols will be introduced in the duct, downstream of the fan and upstream of the proximal sampling station. Bioaerosol introduction will take place over the course of 20 minutes.
- Sampling will take place within the duct upstream (proximal) and downstream (distal) of the device over the duration of the 20 minute introduction as outlined in Table 1 in Appendix 2. Refer to Appendix 3 for sampling methodology.

Similarly, if the client decides to proceed with assessment of the Air Knight® air purification device against ATD, the same methodology outlined above would be used i.e. 3 control runs with the device turned off and 3 test runs with the device turned on.

- ATD will be introduced in the duct, downstream of the fan and upstream of the proximal sampling station. ATD introduction occurs over the course of 20 minutes.
- Sampling will take place within the duct upstream (proximal) and downstream (distal) of the device over the duration of the 20 minute introduction. Refer to Appendix 3 for sampling methodology.

(c) Proximal Sampling in-duct, Distal Sampling in chamber (effect over time)

The chamber is a purpose built ASTM standard chamber of 28.6 m^3 capacity. It is constructed from stainless steel with all materials complying with low VOC emission requirements. The air change rate can be controlled within a range from 1 to 30 Air Changes per Hour (ACH). Temperature and humidity levels can be controlled across a wide range at all ACH rates. AHG recommends that the test conditions will be set at 1.0 air changes per hour (ACH), 21°C and 55% relative humidity.

The filters (MERV 10 filter and 3M Filtrete 2400 MPR filter) which are mounted at the distal end of the test duct will be removed for this these experiments to enable recirculation of air from duct and chamber.

The test duct will be decontaminated between test runs and then HEPA-filtered conditioned air will allowed to circulate in the duct from the adjacent environmental test chamber. The chamber will be then switched to full air dump for 5min to remove any traces of contaminated air prior to commencement of the next run.

The effect of the Air Knight® air purification device will be examined over a time duration decided by Dust Free®. AHG would recommend 4 hours.

AIR KNIGHT® AIR PURIFICATION DEVICE

The following test-run will be performed with the Air Knight® air purification device turned off, in triplicate i.e. 3 control runs, and then with the Air Knight® air purification device turned on, in triplicate i.e. 3 test runs (see Table 2 in Appendix 2 for further details):

- Bioaerosols will be introduced in the duct, downstream of the fan and upstream of the proximal sampling station. Bioaerosol introduction will take place over the course of 20 minutes.
- Sampling will take place within the duct upstream (proximal) and downstream (distal) of the device over the duration of the 20 minute introduction and then every 30min up to 4 hours, as outlined in Table 2. Refer to Appendix 3 for sampling methodology.
- Surface samples will be taken before, after introduction and at the end of each test run as required. Surface samples will be taken in duplicate from 10x10cm areas on the chamber floor.

3. Costs

- **Chamber Daily Rate: €1,500 per day**
- **Assays/Analysis: See table below for cost per sample.**

	Airborne	Surface
Bacteria	€65	€65
Virus	€65	€65
Allergen	€80	€80
Fungi	€65	€65

In order to manage the costs of the project, we are happy to discuss carrying out a preliminary assessment if required. However we would obviously recommend that any data used for marketing claims be generated in triplicate in order to establish its scientific integrity.

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Appendix 1 – Test Items

The species of virus, bacteria and fungus chosen for this study are intended to act as indicator organisms as to how, in general, other species from these micro-organism classes may behave.

Viruses: Bacteriophages are viruses which cannot replicate outside their host bacteria and include coliphages which specifically infect strains of *Escherichia coli* (E.coli). Coliphages have been used as surrogate viruses in numerous studies relating to viral contamination of air, water and food as they mimic the behaviour of pathogenic viruses, while being harmless to humans and animals. The MS2 coliphage (Leviviridae family) is a bacteriophage which specifically infects E. coli (Migula 1895) and is a non-enveloped ssRNA icosahedral virus with a diameter of 25-27nm (0.025-0.027μm). MS2 coliphage is similar in morphology to Picornoviruses and can persist as an infectious virus in the environment comparable with the most resistant human pathogens in the Picornoviridae family e.g. poliovirus, rhinovirus and enterovirus.

Bacteria: *Staphylococcus epidermidis* (ATCC 12228) is a Gram positive aerobe and is 0.5-1.5μm in diameter. It is a major component of the normal skin and mucosal microflora and is one of thirty-three known species belonging to the genus *Staphylococcus*, the same genus that Methicillin Resistant *S. aureus* (MRSA) belongs to. Approximately 50% of staphylococci levels are in the size range capable of penetrating the lungs. These may pose a human health concern since exposures to staphylococci are known to cause toxic shock syndrome, scalded skin syndrome, and soft tissue infections. *S. epidermidis* is usually non-pathogenic, however patients with a compromised immune system are often at risk for developing an infection. It is a consistently isolated micro-organism, being in the top five detected bacteria from hospital units, offices and homes.

Fungi: *Aspergillus niger* (ATCC 16404) is a ubiquitous soil fungus that is commonly found in indoor environments, hospitals etc. Its spore size is in the range of 4 – 5 μm in diameter and it possesses excellent environmental resistance (due to the production of melanin). *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. According to respiratory deposition calculations for the most obvious breathing patterns in the home environment, 30%-50% of fungal particles would be deposited in the nose and 30%-40% in the alveoli during nasal breathing, whereas 70% would be deposited in the alveoli during oral breathing. As such fungi represent a very important aspect of bioaerosol testing.

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Appendix 2 – Outline of Test Runs

Table 1: Outline of Single Pass Test Runs to be performed with the Air Knight® air purification device

To be done in triplicate, 6 runs in total.

Test Run	Sample details	Sampling Locations
Single Pass Test Run 1	**Air Knight OFF**	
Bioaerosol introduction (MS2 Coliphage and *S.epidermidis* (Duration = 20min)	Air Samples are taken during bioaerosol introduction for 20min	Proximal and Distal samples for MS2 Coliphage and *S.epidermidis* will be taken simultaneously in the duct.
Bioaerosol introduction *A.niger* (Duration = 20min)	Air Samples are taken during bioaerosol introduction for 20min	Proximal and Distal samples will be taken for *A.niger* simultaneously in the duct.
Decontaminate Duct, Dump air		
Single Pass Test Run 2	**Air Knight ON**	
Bioaerosol introduction (MS2 Coliphage and *S.epidermidis* (Duration = 20min)	Air Samples are taken during bioaerosol introduction for 20min	Proximal and Distal samples for MS2 Coliphage and *S.epidermidis* will be taken simultaneously in the duct.
Bioaerosol introduction *A.niger* (Duration = 20min)	Air Samples are taken during bioaerosol introduction for 20min	Proximal and Distal samples will be taken for *A.niger* simultaneously in the duct.
Decontaminate Duct, Dump air		

Note: If Dust Free® wishes to proceed with allergen, ATD will be introduced and allergen will be sampled according to the same time-points as described for bioaerosols in Table 1.

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Table 2: Outline of Chamber Test Runs to be performed with the Air Knight® air purification device *To be performed in triplicate for each device (a total of 6 runs).*

Test Run	Sample details	Sampling Locations
Chamber Test Run 1	**Air Knight OFF**	
Bioaerosol introduction of test panel (MS2 Coliphage, *S.epidermidis* and *A.niger*) Duration = 20min	Air Samples are taken during bioaerosol introduction for 20min Surface samples are taken before and after introduction	Proximal in-duct samples and in-chamber samples will be taken simultaneously. Samples will be tested for MS2 Coliphage, *S.epidermidis and A.niger*
n/a	Air Samples are taken after bioaerosol introduction every 30min. Surface samples are taken at the end of the test or more frequently as required.	Proximal in-duct samples and in-chamber samples will be taken simultaneously. Samples will be tested for MS2 Coliphage, *S.epidermidis and A.niger*
Decontaminate Duct, Dump air		
Chamber Test Run 2	**Air Knight ON**	
Bioaerosol introduction of test panel (MS2 Coliphage, *S.epidermidis* and *A.niger*) Duration = 20min	Air Samples are taken during bioaerosol introduction for 20min Surface samples are taken before and after introduction	Proximal in-duct samples and in-chamber samples will be taken simultaneously. Samples will be tested for MS2 Coliphage, *S.epidermidis and A.niger*
n/a	Air Samples are taken after bioaerosol introduction every 30min. Surface samples are taken at the end of the test or more frequently as required.	Proximal in-duct samples and in-chamber samples will be taken simultaneously. Samples will be tested for MS2 Coliphage, *S.epidermidis and A.niger*
Decontaminate Duct, Dump air		

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Appendix 3- Sampling Techniques and Analysis

Sampling Techniques and Analysis:

airmid healthgroup's testing facility incorporates state of the art industrial hygiene and microbiology laboratory capabilities. Sampling of both air and surface is performed during the testing period. Airborne micro-organisms or allergen are sampled upstream and downstream in the test duct and chamber, as required. Surface swabs are taken at the end of the test run. After sampling, the samples are passed to the microbiology or allergen laboratory for immediate processing.

Viruses:

Air sampling is performed with an SKC Biosampler at a flow rate of 12.5L/min.

Surface sampling: Swab samples are taken from representative areas (10x10cm) of the floor and analysed for the presence of viruses. Samples are stored at 4°C until assay. Samples are serially diluted and viral particles are enumerated using cell culture or plaque assay techniques, after incubation at 36.5°C.

Bacteria/Fungi. Air sampling is performed using a Biostage Impactor at 28.3 L/min flow rate. Samples are collected by impaction onto selective agar plates. Surface sampling: Swab samples taken from representative areas described above are divided up to enable analysis for the presence of target bacteria. All samples are appropriately diluted, incubated at 25°C or 35°C for a specified duration, and enumerated following confirmatory identification tests.

Allergen: Air sampling pumps, calibrated at an airflow rate of 2L/min, are fitted with filter cassettes and glass fiber filters. The filter is removed from the collector post sampling and stored at 2-8°C until extracted and analyzed for allergen levels.