FINAL REPORT

Customer Name	**Dust Free**
Customer Address	**1112 Industrial Drive, Royse City, Texas TX 75189, USA**
Contact	**Gregg Burnett**
Customer PO no.	n/a
Test Requested	To assess the impact of the Air Knight IPG by Dust Free on *Staphylococcus epidermidis* bioaerosol circulated in the duct and chamber
Sample Description	'Air Knight IPG by Dust Free' an in-duct air cleaning device
Number of Samples	2
Date of Receipt	3rd February, 2014
ASC Code	ASC002453
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Contents

Purpose

The purpose of this report is to assess the impact of the Air Knight IPG by Dust Free in-duct air cleaning device on airborne *S.epidermidis* levels in the chamber, when mounted in the adjacent modified ASHRAE 52.2 test duct.

Test Item Description

Two Air Knight IPG by Dust Free in-duct air cleaning devices (IPG device) were submitted to airmid healthgroup (AHG) for testing by Dust Free. The IPG device is comprised of a combination of UV and proprietary technology. At the commencement of the testing, the IPG device was mounted into the modified ASHRAE 52.2 test duct as per Dust Free's instructions. Photographs of the IPG device installed in the test duct are shown in Fig.1 below. Fig.2 shows a photograph of the test chamber and test duct. Fig.3 shows the set-up of the IPG device in the duct and the location of the air baffle and the 200 cfm fan.

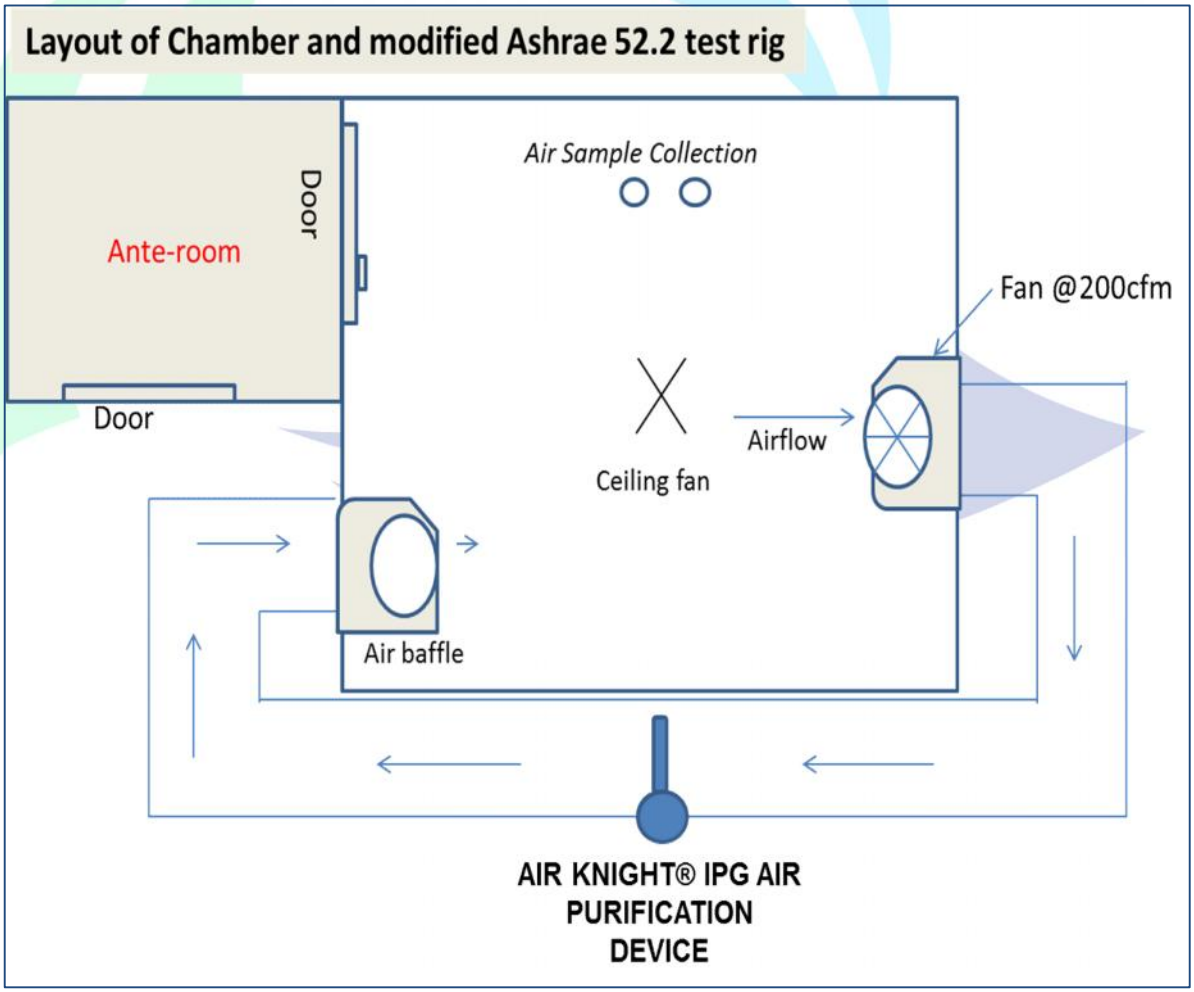
Fig.1 Air Knight IPG by Dust Free mounted in test duct

Outside view by access door

View from inside the test duct

Fig.2 Environmental test chamber and test duct

Fig. 3



Materials:

Staphylococcus epidermidis ATCC12228 was used as the bacterial organism of choice for this study. *S. epidermidis* is a commensal bacterial species commonly found on human skin and is used as a surrogate for more pathogenic *Staphylococcus* species such as MRSA.

At Dust Free's request, the concentration of *S. epidermidis* aerosolized into the chamber and duct for each test run was approximately 3 x 10^6 CFU, which is 20 times more concentrated than normally found indoors.

Sterile Culture Media: Mannitol Salt Agar (MSA)

Cotton swabs and Phosphate Buffered Saline (PBS)

Protocol

Test Conditions:

IPG device testing was conducted in the modified ASHRAE 52.2 test duct and adjacent test chamber as shown in the above figures. The total volume of the duct and chamber is 32.2 m^3. The chamber and duct were pre-conditioned to 21^{o}C and 55% RH prior to commencement of the tests. The chamber was completely shut down during the test so that the air containing the *S.epidermidis* bioaerosol was recirculated from the chamber into the duct at a rate of 6 times per hour. To obtain a rate of 6 air changes per hour, the small fan at the supply side of the test duct (Fig.3) was operated throughout the entire test at a velocity of 0.095 m^3/s which is equivalent to 200 cfm. The fan speed setting was verified by an engineer using a calibrated airflow anemometer, (refer to attached commissioning report).

The bioerosol was circulated from the chamber into the test duct past the IPG device. At the exhaust end of the test duct, an air baffle was installed to ensure there was thorough mixing of bioerosol once it entered the chamber, prior to it being recirculated back into the test duct. The central ceiling fan in the chamber was operated throughout the entire test.

The chamber and test duct were decontaminated between test runs by mopping the surfaces with disinfectant followed by operating UV germicidal lamps, which were mounted in the chamber ceiling and in the test duct, for at least 60 minutes. During this time, HEPA-filtered conditioned air was circulated in the duct from the adjacent environmental test chamber, with the chamber at full ventilation.

IPG Device Tests

Triplicate test runs were carried out for S. epidermidis bioaerosol as follows:

a) 3 inactive runs without the IPG device operating, but with the small fan operating at 200 cfm

b) 3 active runs with the IPG device operating and with the small fan operating at 200 cfm

- In each inactive run (a), the chamber and duct were pre-conditioned and the fans were operated as described in Test Conditions above.
- A background air sample was collected onto a BioStage impactor containing Mannitol Salt Agar (MSA) using a vacuum pump operated at 23 LPM.
- A swab was taken from the chamber floor (10 x 10 cm area) prior to introduction of the *S. epidermidis*.
- Approximately 8 ml of *S. epidermidis* stock was aerosolized into the chamber over a period of 50 mins via a 3 jet collision nebuliser set at 20 psi, in order to achieve the desired concentration of bioaerosol in a steady state.
- Immediately after aerosolization was completed, at t = 0min, two air samples were collected simultaneously onto BioStage impactors containing MSA.
- A further 2 air samples were collected at t = 15 and at t = 30min.
- Surface samples were taken in duplicate from the floor at t = 0 and t = 30min
- The chamber was decontaminated as described in Test Conditions.
- All samples were immediately stored on ice until processed in the laboratory that day.
- In the case of the active runs (b), the test was performed in an identical way to (a) except that the IPG device was switched on at t = 1 min, i.e. 1 min after the 50 min introduction of *S. epidermidis* stock was completed. The IPG device was switched off at t = 30mins and the chamber was decontaminated as previously described.

- Airborne particle counts (0.3-10.0μm) were also monitored throughout the testing to determine if the IPG device had an impact on the particles.

Sample Analysis:

The quantity of *S. epidermidis* detected in the surface swab samples was determined by performing 10 fold serial dilutions of duplicate aliquots of each sample. The serially diluted samples along were then transferred to a petri dish and fixed in molten MSA (cooled to 48ºC). Once the agar had set the petri dishes were incubated at 36.5°C to determine the presence or

absence of viable bacteria in the original swab sample The BioStage MSA plates were also incubated at 36.5°C until evidence of bacterial growth was observed. Colony counts were performed and the results were expressed as colony forming units (CFU)/m^3 for the air samples and as CFU/m^2 for the swab samples.

Calculations

The titre of the *S. epidermidis* stock in CFU/ml was calculated at the start of each test day. The volume introduced into the chamber in the active and inactive runs was also recorded. The airborne titre of *S. epidermidis* in the chamber was then calculated based on the volume introduced for each test run.

The concentration of *S. epidermidis* in each sample taken at t = 0 min, t = 15 mins and t = 30 mins was quantified as CFU/m^3 of air and the average was obtained.
Please Note: The IPG device was activated at t = 1 min, which was immediately after the initial t = 0 samples were taken.

The % Reduction of airborne *S. epidermidis* in the chamber with the IPG device operating was calculated at the 15 and 30min sample points, using the Formula A.

Formula A

$$\% \text{ Reduction} = 100 - \frac{\textit{S. epidermidis}\ \text{CFU/m}^3 \text{ in active runs}}{\textit{S. epidermidis}\ \text{CFU/m}^3 \text{ in inactive runs}} \times 100$$

An unpaired student's *t*-test was performed on the data using GraphPad Prism software to assess if there was a significant difference between the inactive and active test run data at the 15 and 30 minute time-points.

Results & Discussion

Three inactive test runs and 3 active test runs with the IPG device operating for 30 mins were performed using the experimental set-up shown in Fig. 3. For each test run, a predetermined dilution of *S. epidermidis* stock was aerosolized for 50 mins prior to air sampling in order to obtain a steady state in the chamber.

Background samples (an air sample and a surface swab) were taken in the chamber at the start of each test run to ensure that there was no contamination carried over from the previous run. There was no detectable bacteria in the air or on the floor surface of the chamber, indicating that there was no cross contamination between runs and that the decontamination process was effective.

Swabs were also taken from the floor at the 15 mins and 30 mins sample points in the active and inactive test runs. However no bacteria could be detected on the surface at either of these time-points. This is not surprising because the particle size of the *S. epidermidis* aerosol is so small (1 – 3 µm) that it is unlikely to have had time to settle out from the air onto the chamber floor during the relatively short test period.

The initial titre of the *S. epidermidis* stock in the nebuliser was calculated at the start of each test day for the inactive and the active runs performed on that day, as shown in Table 1. The volume of the *S. epidermidis* stock introduced into the chamber was also recorded for each run. From this, an estimate of the airborne titre of *S. epidermidis* aerosol introduced into the chamber and test duct was calculated for the active and inactive runs as shown in Table 1.
As can be seen from Table 1 a similar level of airborne *S. epidermidis* was achieved on each test day in the chamber and test duct, from 2.7 -3.7 x 10^6 CFU/32.2m^3 which is about 20 times more polluted than the average level detected indoors.

Table 1:

Initial titres obtained in the nebuliser for the S. epidermidis (CFU/ml) stock are shown for each test day. The approximate titre of S. epidermidis introduced into the air is shown for each run.

Test Day	Test Conditions	*S. epidermidis* Titre in nebuliser (CFU/ml)	Volume of Stock Nebulised (ml)	Airborne Titre of *S. epidermidis* (CFU/32.2m^3)
1	**Inactive Run 1**	3.3×10^5	8	2.7×10^6
	Active Run 1		8	2.7×10^6
2	**Inactive Run 2**	4.4×10^5	8	3.5×10^6
	Active Run 2		8	3.5×10^6
3	**Inactive Run 3**	4.6×10^5	8	3.7×10^6
	Active Run 3		8	3.7×10^6

Once the introduction of the *S. epidermidis* aerosol was complete, air samples were taken at t = 0 and then at t = 15 mins and t = 30 mins in the chamber while the air was being recirculated at a rate of 6 times per hour through the chamber and test duct. The average airborne titre of *S. epidermidis* in CFU/m^3 was calculated at each sample point for the inactive and active test runs and the results are shown in Table 2. The % Reduction of airborne *S. epidermidis* in the presence of the active IPG device in the test duct was also calculated for the active runs (at 15 and 30 mins) using Formula A. The data is also shown in Table 2.

In the inactive runs there was some natural decay of the *S. epidermidis* observed over the 30min time interval as the initial counts decreased from 4.7×10^4 to 2.1×10^4 CFU/m^3 (Table 2). This is likely due to physical losses in the circulating air and as a result of the nebulisation process itself. In the active runs, the initial counts were 3.8×10^4 CFU/m^3 but this decayed at a faster rate, than in the inactive runs, to 7.3×10^3 CFU/m^3 (Table 2). A % Reduction of airborne *S. epidermidis* of 37.6% was obtained at 15 mins and this increased to 65.2% at 30 mins, with the active IPG device operating in comparison to the inactive runs. This demonstrates that the IPG device started to have a notable effect after just 15min of operation and this increased exponentially to 65% after 30 mins (Table 2).

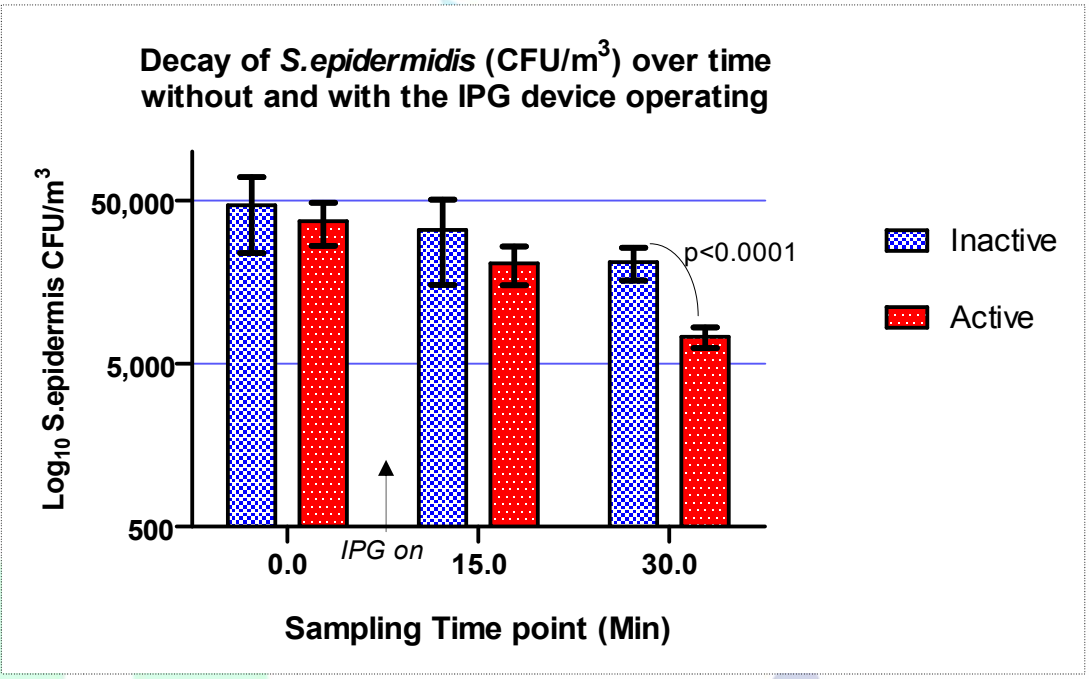
Table 2:

Average airborne titre of *S. epidermidis* (CFU/m^3) and % Reduction of airborne *S. epidermidis* after 15 and 30 min operation of the IPG device is shown.

Test Condition	Sample Time Point (min)	Average Airborne *S. epidermidis* Titer (CFU/m^3)	% Reduction of Airborne *S. epidermidis* in Active runs (%)
Inactive	**0**	**4.7 x 10^4**	**n/a**
	15	**3.3 x 10^4**	**n/a**
	30	**2.1 x 10^4**	**n/a**
Active	**0**	**3.8 x 10^4**	**n/a**
	15	**2.1 x 10^4**	**37.6**
	30	**7.3 x 10^3**	**65.2**

The average decay of the *S. epidermidis* at each sample point is shown graphically in Fig. 4 for the 3 active and 3 inactive runs. As can be seen from the graph, the decay of the bacteria in the active runs was quite marked after 30mins of the IPG device being activated compared to the control. An unpaired students *t* - test on the airborne *S. epidermidis* titers (CFU/m^3) demonstrated a statistically significant difference (t = 6.824 and P = 0.0001) after 30min operation of the IPG device in the active runs (n = 3) compared to the inactive runs (n = 3). The results were not significant at 15 mins.

Fig. 4: The Decay of airborne *S. epidermidis* over time for the inactive and the active runs.



IPG turned on in active runs at arrow shown on graph.

Airborne particle counts were recorded throughout the active and inactive runs. The average airborne particle counts for particle sizes 0.3, 0.5, 1.0, 3.0, 5.0 and 10.0 µm for the 3 active and 3 inactive runs were calculated and are shown graphically in Appendix 1. As can be seen from the graphs the IPG device did not influence the level of airborne particles compared to the inactive control runs, even though it have a reducing effect on the airborne *S. epidermidis*.

Conclusion

The Air Knight IPG by Dust Free was demonstrated to be effective in reducing airborne concentrations of *S. epidermidis* aerosol in the chamber, under multiple pass conditions (i.e. at a rate of 6 ACH) by 65% within 30 mins of operation. It had no effect on airborne particles in the size range from 0.3 - 10.0 µm. The average rate of decay of *S. epidermidis* (CFU/m^3) circulating in the chamber with the IPG device activated for 30min was significantly different ($P < 0.0001$) from the rate of decay detected in the inactive runs. Based on these data, a test run of 30 mins is quite short and a more marked reduction would likely be seen if the IPG device was activated for a longer time interval.

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Appendix 1:

Airborne particle counts/m^3 measured throughout the inactive and active tests