

Bactericidal Properties of Electrically Enhanced HEPA Filtration and a Bioburden Case Study

by

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Abstract

The bactericidal properties of an ionizing electrically enhanced filter (EEF) were evaluated in laboratory conditions using Staphylococcus epidermidis. A test protocol was developed which enabled comparison of bacteria survival on control (conventional) and EEF filters. The results indicate that almost 100% of the bacteria were killed by the EEF. A field evaluation of the EEF technology was also conducted in a manner such that the EEF could be directly compared to conventional HEPA filter technology. The field study indicates that the EEF resulted in about 55-75% lower bioburden as compared to conventional filtration. Further, the bio burden in the Class 10K room (at rest), with EEF, was equivalent to that of a Class 100 room.

1.0 Background

The fundamental purpose of cleanrooms in the pharmaceutical, medical and biotechnology industries is to control the amount of bio burden due to internal operations and due to transport from the air. Cleanrooms in these industries are classified and specified according to the same cleanroom standards (e.g. Federal Standard 209E) as in other industries, since it is assumed that the clean classifications, in terms of particle (viable or non viable) concentrations will generally correlate to

concentration of viable microorganisms. This correlation may not always hold. Thus the concentration of viable organisms is also directly measured - both at the work surfaces (or at the process) and in the air.

The FDA has specific requirements and guidelines for bio burden for various pharmaceutical operations and processes. Similarly, the European Union's GMP guidelines give specific recommended limits for microbial contamination for each class of room. A cleanroom that meets the particle concentration requirements, but does not result in the desired level of bio burden, will clearly be inadequate.

One of the main obstacles in maintaining the required bio burden levels, is that the measurement of bio burden is time consuming. Typically, bio burden measurement involves sampling, incubation and counting of colonies. Recently, however, UV fluorescence (cf. Seaver and Eversole [1] Pinnick et al. [2]) technology has made it possible to achieve "real time" monitoring of particles of biological origin. This technology will find increasing use in the real time monitoring of air in hospitals, clean rooms and military nuclear, biological and chemical (NBC) warfare protection systems - as a real time supplement to the standard methods of determining bio burden. *As this happens*

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more attention will be focused on clean room contamination control systems - currently mainly mechanical filtration.

One problem with mechanical filters is that under certain conditions common bacteria caught on the filter can start growing on the filters, grow through the media and start shedding into the room [5,6,7]. The well known case of the *Legionnaire's* outbreak at the veterans convention in Philadelphia has been attributed to this phenomena. In that case the filters were supposedly in a wet state. Generally, it is accepted that bacteria is difficult to grow on clean glass fiber filter media, used in HEPA filters, under normal humidity conditions. However, since the function of these filters is to capture all particulate contamination, filters eventually get dirty. The experiments conducted by Jaisinghani et al. [4,9] show that very little contaminant is needed for growth of *Staphylococcus epidermidis* and *Escherichia coli* on HEPA glass filters. In their experiments Jaisinghani et al. [4,9] found that very little of the applied *E. coli* survived on the clean glass filter after four hours of air flow, keeping in mind that *E. coli* is not a hardy organism. Next about 1 gm of colloidal kaolin was added to the *E. coli* solution that was to be aerosolized. This time the recovery of *E. coli* was about 10^4 - 10^5 CFU/square inch of the filter media - at 45% RH!. Similar tests with *S. epidermidis* recovered a little more *S. epidermidis* than with *E. coli* - without the colloidal kaolin, due to the more hardy nature of *S. epidermidis*. With 1 gm of colloidal kaolin in the 25 ml *S. epidermidis* solution (in tryptic soy broth) the recovery of *S.*

epidermidis was about 10^5 - 10^6 CFU/square inch of filter media. Tests with air flow continuing for 7 hours (following the aerosol) did not result in any significant reduction in bacteria recovery.

Consequently, even in normal environments bacteria can survive or grow on the filters. As the trend towards using HEPA clean room filters for longer periods (based on pressure drop constraints) continues, the possibility of bacterial growth on the filter, and thus the rise in the air bio burden, also increases.

Virus particles can be much smaller - as small as 2 nm. Although it is reasonable to expect that most viruses will be present as aggregates or will be attached to other particles, a small fraction may be expected to be in the fundamental state - as a single virus - and thus can penetrate HEPA filters. Depending on the virus, this may be dangerous or detrimental to the product in the cleanroom. Most viruses are not expected to survive for long in the absence of moisture. However, some studies (cf. [3]) show that the common cold virus (*rhinovirus*) may survive for as long as 2-4 hours in normally low humidity winter indoor environments. Dr. Elliot Dick's (cf. [3]) work at the University of Wisconsin has shown that the primary transmission of the common cold (in adults) occurs through air - and not through direct contact.

This paper discusses the bactericidal properties on an ionizing electrically enhanced filtration technology, and how this affects bio burden in cleanrooms.

2.0 EEf Technology

Jaisinghani [4,11] has played a significant Bactericidal Properties of EEf....
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role in the development and commercialization of EEF technology. The most recent version (see Figure 1) of this technology maintains the filter under an *ionizing* (as opposed to a simple electrostatic field) field. Another higher intensity ionizing field charges incoming particles, stabilizes the electrical fields and increases the safety and reliability, by ensuring that no spark over can occur towards the filter. This method provides two fundamental benefits:

1. *Bacteria are killed as they pass through a first high intensity ionizing field and then killed as they are subjected to continuous ion flux when they are trapped on the filter. This inhibits survival and growth of bacteria on the filter.*
2. *The same ionizing fields enable penetration reduction by about three orders of magnitude.*

Since the cost of the additional electrical components is partially offset by the increase in filtration performance (either higher flow at the same pressure drop and filtration efficiency or lower pressure drop at the same flow and efficiency, as compared to mechanical filtration of the same size) this is a highly cost effective way to achieve a higher level of bio burden control. Figure 2 shows the ratio of the fractional filter penetration without electrical enhancement, P_0 , to the penetration of the same filter, with electrical enhancement, P_e . [Penetration is 1 - fractional efficiency, i.e. the fraction of particles not captured by the filter.] The higher the penetration ratio, P_0/P_e , the higher is the effectiveness of the EEF technology. The ionizing EEF achieves about three orders of magnitude improvement, at 0.3

Um, in filtration performance at a very high flow velocity (about 600 fpm). A commercial filter unit based on this ionizing EEF technology is currently being marketed by Technovation, Midlothian, VA.

It should be noted that older electrically enhanced filter technology (cf. Bergman et al [10], and Jaisinghani et al. [11]) do not maintain the filter under an ion flux and hence do not have the potential to kill

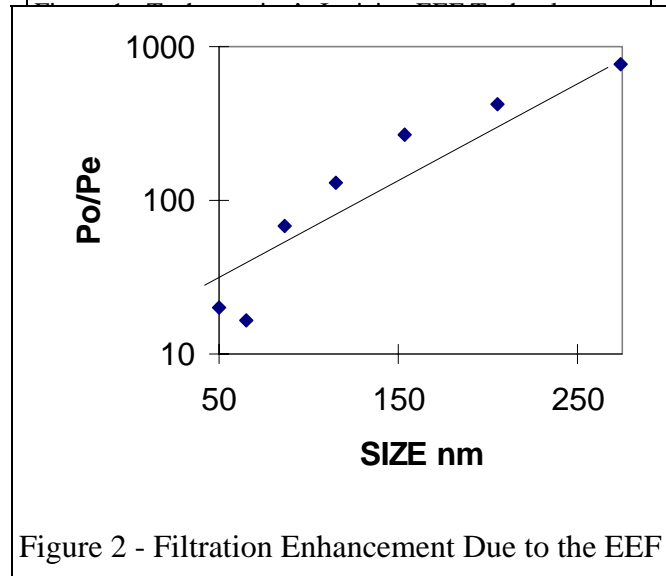
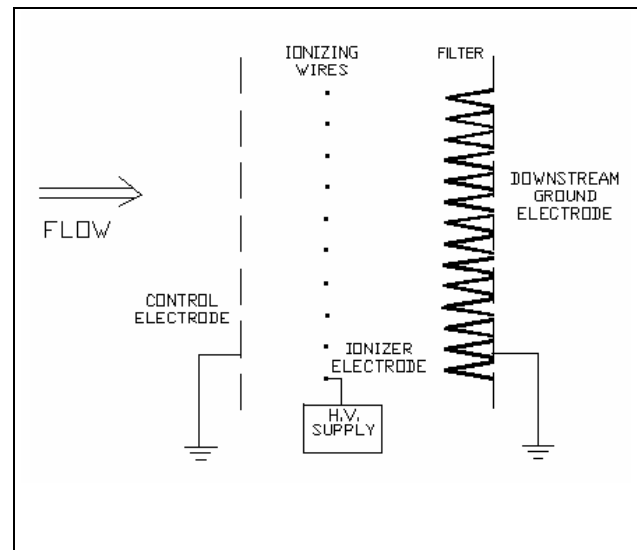


Figure 2 - Filtration Enhancement Due to the EEF

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bacteria (simple electrostatic fields are not effective in killing bacteria¹).

3.0 Laboratory Evaluation

3.1 Experimental methods

Staphylococcus epidermidis was grown in Tryptic Soy Broth (TSB) to a concentration of 3×10^9 colony forming units (CFU)/ml. The culture was lyophilized in Wheaton vials in 5 ml aliquots for a total of 1.5×10^{10} CFU per vial. All vials were stored in a desiccator at 4-6 C.

Pleated 15.24cmx15.24cmx5cm (6"x6"x2") deep filters were first coated with kaolin and TSB using an Aztek airbrush. The airbrush cup was filled with 1g kaolin suspended in 25ml TSB and sprayed onto a filter inside a laminar flow hood and allowed to air dry before being used. The pleated filters were placed in a miniature prototype electrically enhanced filtration (EEF) device. One vial of lyophilized *S. epidermidis* was resuspended with 1 ml of sterile distilled water. A small aliquot of this suspension was serially diluted ten-fold to 10^{-8} and plated on Columbia Blood Agar (CBA) plates to confirm the initial viable concentration of bacteria. The rehydrated culture was then sprayed onto the filter using a Meinhard nebulizer, which was placed 8 inches from the center of the filter.

A control assay was performed to determine the amount of viable

S. epidermidis that collects on the filter, without applying high voltage to the EEF prototype. The bacteria were sprayed onto the filter as previously described, and the temperature and humidity were monitored every 15 minutes for four hours or seven hours during which the air flow was on. The relative humidity was held between 45-55% using a Kaz steam vaporizer. At the end of each control run three pieces of the filter were extracted using a sterilized scalpel and forceps. The pieces of filter were approximately 1 square inch on the face of the filter, which when unfolded measured approximately 28 square inches of filter material. Filter pieces were removed from the center of the filter, directly above the center, and directly below the center.

The samples were cut into small pieces and placed into 10 ml of sterile phosphate buffered saline (PBS) pH 7.4 in a Nasco Whirl-Pak bag. The bags were then processed in a Tek Mar Stomacher Lab-Blender 80 for one minute. Each sample was then serially diluted ten-fold to 10^{-2} , 10^{-3} , and 10^{-4} , then spread on CBA plates to determine the number of viable bacteria per sample filter piece.

The bactericidal effect of the EEF was tested as described above by means of applying high voltage using an Acopian High Power Supply. In addition to monitoring the temperature and humidity, the current was also monitored at fifteen minute intervals during the 4 or 7 hour period of air flow with the applied high voltage on. Filter samples were processed as described above.

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¹ Based on unpublished work using *E. coli*, by Dr. Elliot Dick at the University of Wisconsin.

3.2 Results and discussion

The results are summarized in Table I. In the absence of any voltage applied to the EEF unit (i.e. control tests), bacteria could be recovered from 1 sq. in of filter in the range of 1×10^5 CFU to 2×10^6 CFU. Counts greater than about 3×10^6 CFU were too crowded to be accurately counted and were considered to be TNTC (too numerous to count). When high voltage was applied for 4 hours the majority of the bacteria were killed. The kill rate increased with increased voltage or the first applied field strength (applied voltage divided by the distance of the ionizer wires from the control ground electrode - see Figure 1), V/d1. At a field strength (V/d1) of 4.2 kV/cm, there was no growth after 24 hours of incubation. After 48 hours, there was either no growth or small colonies grown. These small colonies were identified as *S. epidermidis*, and were identical in biochemical profile as the isolate used in the tests. It was concluded that 4 hours at 4.2 kV/cm (V/d1) did not completely kill all the *S. epidermidis*. If the bacteria were not all killed, some of them were damaged sufficiently so that no growth or very limited growth could occur after 24 hours incubation. When the ionizing time was increased to 7 hours, over 99% of the bacteria (as compared to the control) were killed - even with 48 hour incubation time period.

When the applied field strength, V/d1, was increased to 4.5 kV/cm or higher, no growth occurred on any of the filter pieces except for one experiment. This exception

may have occurred because the starting dose of bacteria for this experiment was 3 times higher than for the control and up to 10 times higher than for any other experiment. Nonetheless, there were still 3-4 logs of killing using an applied field strength, V/d1 of 4.5 kV/cm or higher, as compared to the control experiments.

In conclusion, increasing the voltage or applied field strength was more effective in terms of killing bacteria than increasing the ionizing time. Field strengths (V/d1) of 4.5 kV/cm or greater resulted in approximately 100% killing of the bacteria on the filter within 4 hours. Thus, field strength (V/d1) of 4.5 kV/cm would be considered optimal (in terms of 4 hour killing efficiency) for bactericidal effect on *S. epidermidis*. However, it should be noted that, in practice, bacteria caught on the filter are held within the ionizing field for an almost infinite amount of time, thus receiving an almost infinite radiation dosage. Hence, in practice, the killing efficiency should be higher even at lower field strengths. Further, note that the conclusions regarding the effect of the applied field strength on the bactericidal effect are only valid for the dimensional gaps (Figure 1), used in this study. Similar results were obtained using *Escherichia Coli* in a previous study conducted with the EEF at the University of Wisconsin. In this study the aerosol was fed in a highly wet state - causing increased current consumption.

Table I - EEF Bactericidal Test Summary using *S. epidermidis*

FILTER	INCUBATION TIME	EEF EXPOSURE TIME	EEF FIELD STRENGTH	AVERAGE COLONIES	COMMENT
control/EEF	hours	hours	(v/dl) kv/cm	#/sq inch	
control	24.00	0.00	0.00	1.00E+06	No Additional Growth
control	24.00	0.00	0.00	1.02E+05	After 24 Hours
EEF	24.00	4.00	4.64	0.00E+00	100% KILLED
EEF	24.00	4.00	3.99	3.44E+02	99.93% KILLED
EEF	24.00	4.00	4.24	0.00E+00	100% KILLED
EEF	24.00	4.00	4.50	0.00E+00	Some Growth
EEF	24.00	4.00	4.20	0.00E+00	After 48 Hours
EEF	24.00	4.00	4.20	6.26E+03	98.75% KILLED
EEF	48.00	7.00	4.20	5.44E+02	99.9% KILLED
EEF	48.00	4.00	4.80	2.16E+02	99.95% KILLED
EEF	48.00	4.00	4.20	3.51E+03	99.3% KILLED

4.0 Field Bio Burden Study

The efficacy of the Technovation’s Model 1001 EEF (see Figure 3), under actual field conditions, was evaluated at and by Encelle, Inc., Greenville, NC. Encelle had four conventional HEPA fan filter units (FFUs) installed in their tissue culture laboratory, prior to replacing these with one Model 1001 EEF. One model 1001 provides HEPA filtered air at about the same total flow (approx. 4250 m³/h (2500 scfm) in this case). This allowed direct with respect to conventional filters, in terms of bio burden in the room, under field conditions. Table II below shows the size and flow rate of the EEF and the conventional FFU HEPA filters:

Encelle personnel had monitored the bio burden in the air using slit to growth medium samples, prior to installation of the Model 1001 EEF. This was continued after installation of the Model 1001 EEF. The bio burden obtained in the air was termed “air

Table II - Physical Comparison of EEF HEPA Model 1001 with respect to FFUs

PERFORMANCE VARIABLE	MODEL 1001 EEF	TYPICAL FFU
Max Flow, m ³ /h (scfm)	5097 (3000)	1359 (800)
Weight, kg (lbs.)	81 (179)	29.5(65)
Flow/weight m ³ /h-kg (scfm/lb)	62.9 (16.76)	46 (12)
Size (Volume) m ³ (ft ³)	0.62 (21.9)	0.23 (8)
Flow rate/Volume (size)1/h (1/min)	8221 (137)	5908 (100)

quality”. The air quality was measured with personnel and without personnel in the room. The average of this (termed “overall score”) was also computed.

The surface bio burden was measured, by means of growth medium plates, only after installation of the Model 1001 bench level.

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Hence, no comparable measurements were available with the conventional FFU system.

4.1 Sample Analysis -Encelle “Score” - Weighting Scheme

The samples were incubated for 24, 48 and 72 hours. After each period the samples were inspected (colonies counted) for growth. If after 72 hours there was no growth (colonies) then the weighting factor was zero. Each colony counted after 72 hours of incubation were assigned a weighting factor of 1. Similarly, each colony counted after 48 and 24 hours were assigned weighting factors of 2 and 3 respectively. Hence, the smaller the score the lower the bio burden. Each measurement sequence (update) involved multiple samples. This score is only a relative number.

4.2 Bio Burden Results

Figure 4 shows the Encelle score (weighted bio burden) at the bench surface . Since there was no data prior to the installation of the Model 1001 EEf, no conclusion regarding the relative effectiveness of the EEf can be made from this data. However, it is interesting to note that the surface Encelle Score temporarily increased after the HVAC maintenance and much more significantly after installation of a new air conditioning system.

Figure 5 shows the air quality (bio burden) results. In this case it is clear that the installation of the Model 1001 significantly reduced the bio burden (as compared to the four FFUs). The range of reduction was about 60-75% as compared to the FFU bio burden level (not including the AC maintenance data) . This data also shows that the Encelle score increased after the



Figure 3 Model 1001 EEf filter

HVAC maintenance and installation of the new air conditioning unit.

4.4 Follow up Bioburden monitoring

After the above initial direct comparative testing, in 1998 Encelle switched to measuring bio burden in terms of concentration - CFUs/ft³. Table III below shows the bio burden of the average 1998 values for the Encelle Class 10K laboratory as compared to the USP and European Union recommended bio burden for various class cleanrooms.

Clearly, from a bio burden perspective Encelle's Class 10K room performs at a level equivalent to a Class 100 room (without personnel - i.e. at rest) - without incurring the higher cost associated with building a Class 100 room. With personnel the Class 10K room performed as a Class 1000 room. From the surface bio burden level the Encelle Class 10K room performs at a USP Class 100 level.

Since the above conclusion is based simply on the air transported bio burden, most of this benefit should be attributed Bactericidal Properties of EEf....

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to the of the EEF filter system.

5.0 Conclusion

Both laboratory and field evaluations have demonstrated the bactericidal effects of the EEF in terms of reducing the airborne bio burden. In higher than Class 100 environments, the EEF provides the required air flow at HEPA particle filtration

efficiency, but with lower bio burden than conventional filtration systems. In Class 100 or better aseptic processing cleanrooms the EEF should find use as a the first stage HEPA filter component of a double HEPA filter system (including terminal HEPAs), thus providing a higher level of safety and quality assurance.

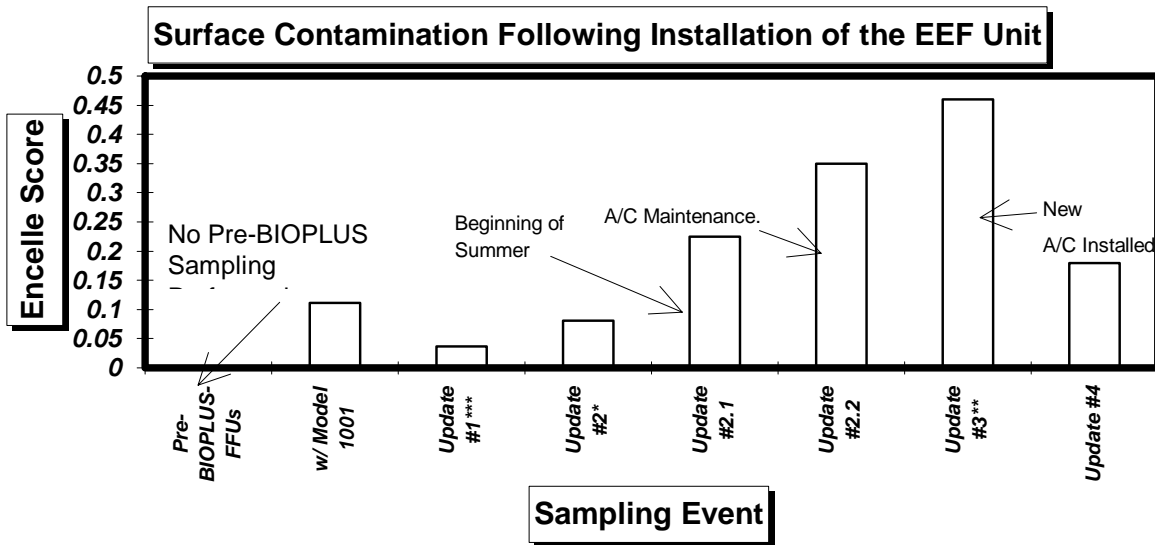


Figure 4 - Encelle Surface Score

Table III Bio burden (CFU/ft³) in Encelle's Class 10,000 room compared to recommended cleanroom values

Fed Std 209E Class	USP airborne	USP surface	EU airborne	Encelle* out of process	Encelle* in process	Encelle* Surface
100,000			<5.67			
10,000	<2.5		<2.83	0.021	0.392	0.256
1,000	<0.5					
100 in process	<0.1	<3	<0.028			
100 at rest			<0.283			

* Average Encelle 1998 bio burden

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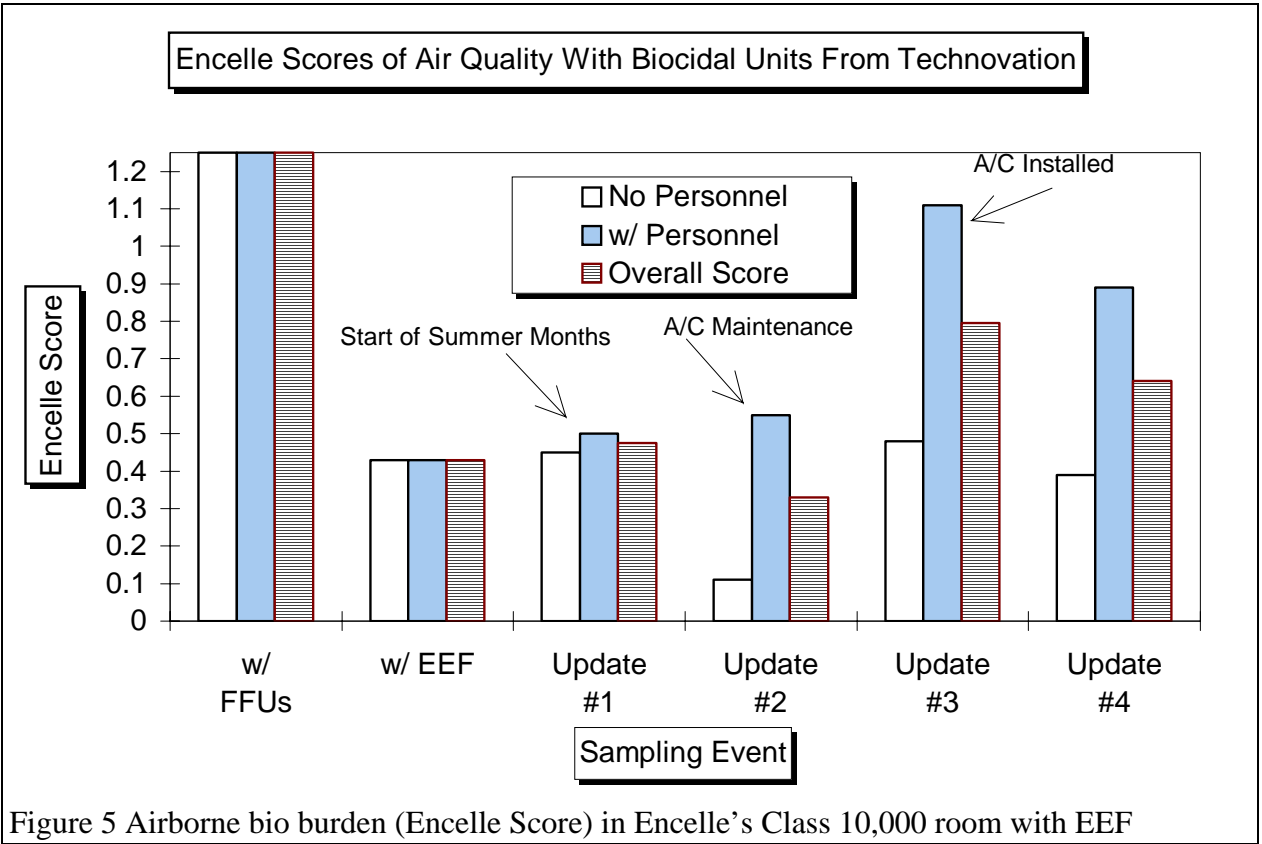


Figure 5 Airborne bio burden (Encelle Score) in Encelle's Class 10,000 room with EEF

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