

Technologies for Monitoring and Control of Airborne Bio Burden/Microorganisms

by

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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. This article reviews technologies for the monitoring and control of bacteria. The scope is restricted to microorganisms in the cleanroom air.

MONITORING TECHNOLOGIES

One of the main obstacles in achieving 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. The results are reported in terms of CFUs (colony forming units) per cubic feet of air.

There are different bio aerosol sampling methods in use. The ideal sampling method must not only recover or collect all the aerosol efficiently, but must preserve the "culturability" or survival of viable microorganisms. There are predominantly two different types of sampling devices - devices that impinge the aerosol on sterile collection plates and devices that collect the aerosol on sterile filters. Collection can involve collection aids. For example the impinger plates can be coated with agar. Additionally gelatin filters are available for analysis of airborne microbes.

In an impinger, a fixed volume of air is sampled and impinged on a collection plate. Typically slit to agar samplers are used. This is basically an impactor that impinges particles greater than a certain size (dependent on the slit size, the distance to the collector and the velocity) on to a growth medium plate. In the AGI-30 impinger, the aerosol is collected in an autoclaved flask typically filled with 20 ml of sterile de-ionized water with 0.01% Tween and an anti foaming agent. Similarly, a fixed volume of air is

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sampled through sterile collection filters, which are then eluted typically using sterile deionized water. The collected suspensions are then serially diluted and then plated onto the growth medium.

Different sampling methods give different results with different types of bio aerosols. For example Lin and Li [12] have shown that the widely used AGI-30 glass impinger, developed originally for bacteria sampling, has a total recovery ranging from 4%-24% for *Penicillium citrinum* spores. Apparently, the stress, due to collection, reduces the total cultured recovery. As shown by Lin and Li [12], different total recovery can be expected for bacteria and spores.

The factors that may affect recovery include aerosol concentration and composition, inlet orientation, aerosol charge, particle desiccation and shear forces, wind speed, particle breakup, sampling time and sampling flow rate. Lin and Li [12] measured the Relative Survival (RS) and the Total Survival (TS) for the AG-30, membrane filter (Nuclepore) and gelatin filters (Sartorius) using *P. citrinum* with a geometric mean diameter of 2.32 μm and geometric standard deviation of 1.26. The RS is the recovery of the *P. citrinum* as compared to the AG-30 and TS is the recovery compared to the amount of aerosol injected into the sampler (as measured by a particle counter). Figures 1 a) b) and c) show the results obtained for the different methods.

Other methods involve setting a growth medium plate on a work surface for a fixed amount of time. After this "collection" time, the sample is incubated for at least 24 hours. After the incubation period the number of colonies is counted and the CFU/ volume of air is calculated. Basically, if a collected particle is viable, then it will form a colony, which is easily recognized or observed.

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. Typically lasers with wave lengths of between 240 - 550 nm are used for this purpose. Pinnick et al. [2] used a pulsed laser system at 266 nm. This research work is based on the fact that several primary compounds in cells, such as amino acids tryptophan (350 nm) and tyrosine (300 nm), reduced nicotinamide adenine dinucleotides (NADH - 450 nm) and flavins (540 nm) exhibit UV fluorescence. This makes it possible for instruments based on this technology to distinguish between non biological and biological particles and simultaneously to measure the particle size. More recent work shows that this technology has the potential to distinguish between at least some type of bacteria.

One commercial instrument (TSI's (St. Paul, MN) FLAP2) is already available. TSI claims that this instrument measures "the intrinsic fluorescence of particles containing living organisms", and therefore is able to distinguish between particles containing viable organisms and other non viable particles. While this technology may not result in the same bio burden, as obtained by the traditional sampling and incubation methods (in terms of colony forming units (CFUs) per unit volume of air), it will find increasing use in the real time monitoring of air in hospitals, clean rooms and military nuclear,

Figures 1 a, b and c - Performance of AG-30, Nuclepore and gelatin filters. *From J. of Aerosol Science and Technology, vol 28, #6. Courtesy of Elsevier Science Inc. and Am. Assoc. of Aerosol Research.*

biological and chemical (NBC) warfare protection systems - as a real time supplement to the standard methods of determining bio burden. *As this happens more attention will be focused on clean room contamination control systems - currently mainly mechanical filtration.*

CONTROL TECHNOLOGIES

MECHANICAL FILTRATION

This is *the* predominant technology currently in use in terms of controlling air borne particles - whether biological or not - in clean rooms and indoor air quality applications. Mechanical HEPA filters form the mainstay of clean room contamination control systems. Since bacteria are much larger than the sub micrometer size particles that are well controlled by HEPA filters (these are at least 99.97% efficient at 0.3 μm), they are easily removed by the clean room HEPA filters. Virus particles on the other hand 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. Depending on the virus, this

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may be dangerous or detrimental to the product. Most viruses are not expected to survive for long in the absence of moisture. However, some studies 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 - including kissing!

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. 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. Many filtration engineers claim that bacteria growth is not a problem "unless bacteria bring with them their own food". However, since the function of these filters is to capture all particulate contamination, filters eventually get dirty. Additionally bacteria and other microbes are often attached to other dirt particles. This dirt becomes food for the bacteria. 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. The following 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.

Clean 6"x6" x2" deep glass mini pleat (with ribbon separators) filters were subjected to *E. coli* aerosol. Following this the air flow (without the aerosol) was continued for 4 hours. The air temperature was maintained around 70⁰ +/- 5⁰ F with a relative humidity (RH) at 50% +/- 5%. Then the filters were cut up, bacteria extracted, plated on growth medium and then incubated for 24 hours. Very little of the *E. coli* survived on the clean glass filter, 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 10⁴ - 10⁵ CFU/square inch of the filter media. Similar tests with *S. epidermidis* recovered more *S. epidermidis* than with *E. coli* even 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 10⁵ - 10⁶ 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. Keeping in mind that the dirt holding capacity of a filter of this size (to a point such that the filter pressure drop increases to double the initial value) is about 40 g, *clearly very little (1 g colloidal kaolin) contaminant is necessary for sufficient growth or survivability of bacteria.* This work suggests that we should expect common bacteria to survive and grow on glass HEPA filters, even under normal temperature and RH conditions. Thus these filters, instead of being contamination control devices, can become the cause of the problem!

MECHANICAL FILTERS WITH BACTERICIDES

In order to reduce the possibility of bacterial growth on filters, some HEPA filter manufacturers offer filter media treated with bactericidal chemicals. These chemicals can be effective in killing many common indoor microorganisms - provided they are in contact with the filter (Hoenig et al. [5] and Rhodes et al. [6]). Once a layer of contaminant builds up on the filter, the bactericides are usually ineffective (Tolliver [7] and Hoenig et al. [5]). Another concern regarding biocide impregnated filters is that many of the biocides (especially fungicides) are carcinogenic and thus filters need to be handled with care. Although most of the chemicals used have low vapor pressure, there may be some concern with some types of biocides stripping off into the air flow.

IONIZING ELECTRICALLY ENHANCED FILTRATION (EEF)

Jaisinghani [8,9] has played a significant role in the commercialization of EEF technology. The most recent version (see Figure 2) 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 1997 R&D 100 award winning 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 ionizing radiation when they are trapped on the filter. This inhibits growth of bacteria on the filter.*
2. *The same ionizing fields enable the filtration performance (penetration reduction) to increase by about three orders of magnitude.*

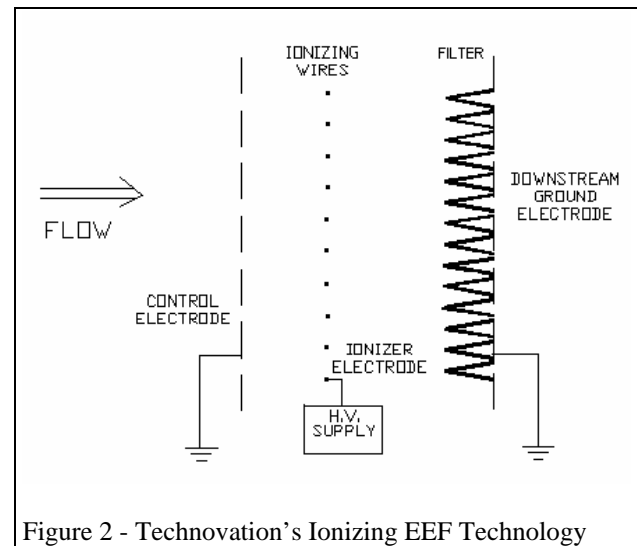


Figure 2 - Technovation's Ionizing EEF Technology

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 3 shows the ratio of the fractional filter penetration (for various particle sizes in nanometers i.e. a thousandth of a micrometer) 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 μ m, in filtration performance at a very high flow velocity (about 600 fpm). A commercial air handler unit based on this ionizing EEf technology is currently being marketed by Technovation, Midlothian, VA.

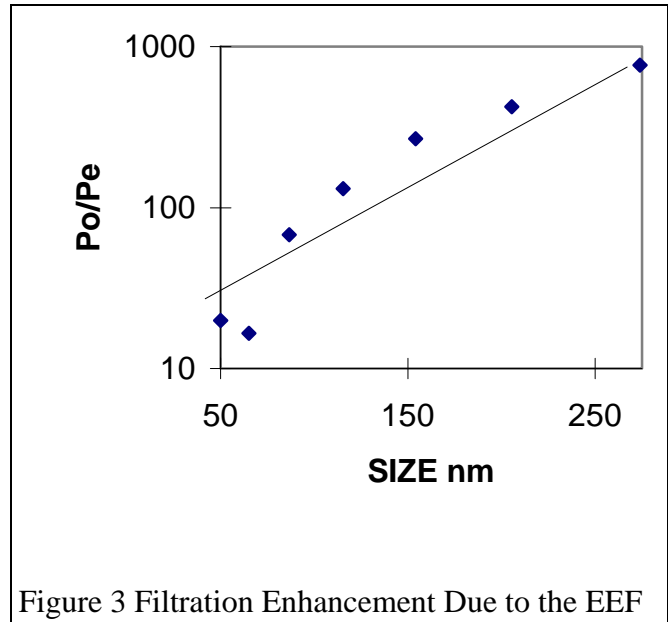


Figure 3 Filtration Enhancement Due to the EEf

The bactericidal properties of the Technovation's EEf were evaluated under laboratory and field conditions. Laboratory tests similar to the ones described above (Mechanical Filtration) were conducted with *S. epidermidis*. The results (Table I) show that the EEf killed almost 100% of the bacteria as compared to the control tests (without electrical enhancement). The viable *S. epidermidis* were reduced from about one million CFU/square inch of filter media, to almost zero! Similar results were obtained with *E. coli*.

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

FILTER	INCUBATION TIME	EEf EXPOSURE TIME	EEf FIELD STRENGTH	AVERAGE COLONIES	COMMENT
control or EEf	hours	hours	(v/d1) 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

The efficacy of Technovation's Model 1001 EEF, in terms of reducing bio burden under actual field conditions was evaluated by one of the users of the Model 1001 filter - 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 also provides HEPA filtered air at the same total flow (approx. 4250 m³/h (2500 scfm) in this case). This allowed direct evaluation of the effect of EEF on the bio burden in the room, under field conditions

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

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. The bio burden was reported as a complex weighted variable - the Encelle score. The Encelle Score or weighting scheme is as follows:

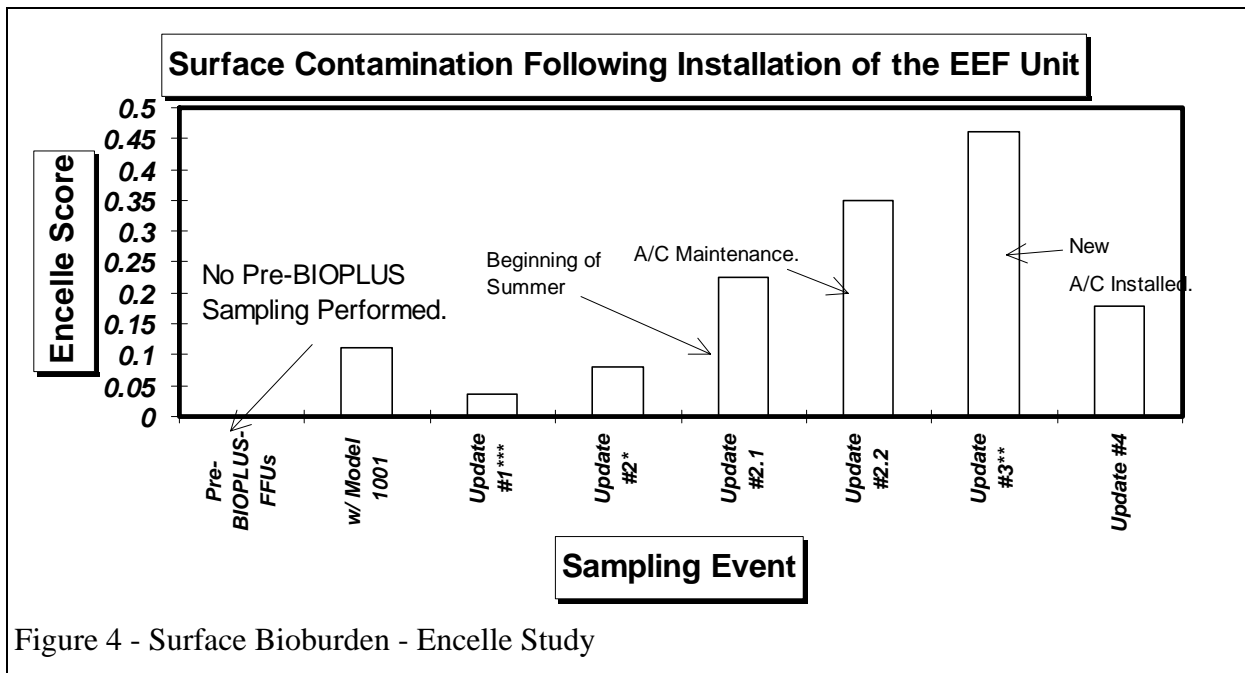


Figure 4 - Surface Bioburden - Encelle Study

CFUs observed after 24 hours incubation: weighting factor = 3

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CFUs observed after 48 hours incubation: weighting factor = 2

CFUs observed after 72 hours incubation: weighting factor = 1

Hence, the smaller the score the lower the bio burden. Each measurement sequence (update) involved multiple samples. This score is only a relative number.

Field Bio Burden Results

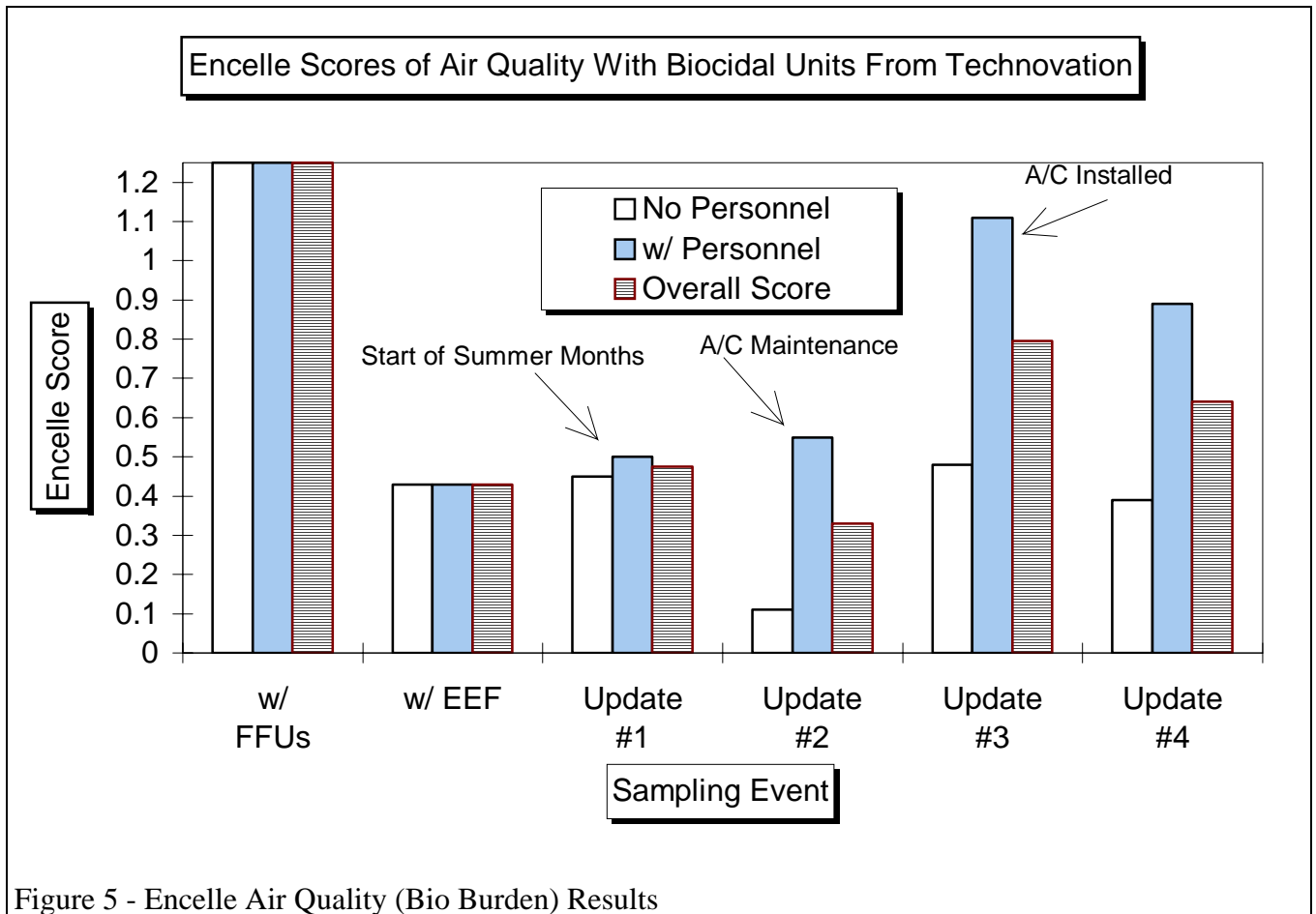
Figure 4 shows the Encelle score (weighted bio burden) at the bench surface. Since there was no data prior to the installation of the Model 1001 EEF, no conclusion regarding the relative effectiveness of the EEF can be made from this data. However, it is interesting to note that the surface Encelle Score 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 bio burden reduction, due to the EEF, was about 65% from the FFU bio burden level. Additional data since then has shown that the bio burden in the room, with the EEF, is now even lower. This data also shows that the Encelle score or bio burden increased after the HVAC maintenance and installation of the new air conditioning unit. This suggests that the room monitoring must be more intense after any work is done to the duct system. It would be interesting to conduct a similar research study before and after the periodic HVAC system validation, as required by cGMP procedures in the pharmaceutical industry.

UV RADIATION

UV radiation is commonly used for sterilization applications. Recently, this technology has been incorporated into fan powered filtration and HVAC duct systems. In combination with filtration, Sarpino and Jensen [10] have shown that this technology, if properly used, can achieve inactivation/kill efficiencies in the high 90% range. The UV radiation requires a minimum residence time (radiation dosage), and therefore the equipment is large - unless lamps are simply used indiscriminately - simply to be able to claim that UV lamps are used. Additionally, the cost of the UV lamps cannot be offset by other advantages, such as improved filtration performance, as in the case of the EEF. However, this technology should find application in critical medical and other specialized applications.

Another form of UV technology is the direct use of UV lamps in quarantine and other infectious disease control rooms. Miller-Leiden et al. [11] have studied the effectiveness of UV lamps in rooms. In order for the UV radiation to not be detrimental to occupants in the room, the UV lamps need to be ceiling or wall mounted with some sort of shielding such that the radiation at the personnel level is within safe limits. This limitation significantly lowers the potential of this technology. In time based tests Miller-Leiden et al. [13] achieved 30-100% reductions with *Micrococcus luteus*, *Bacillus subtilis* and *E. coli*. These methods should find application as supplementary control methods.



SUMMARY

In summary, recent research and development has resulted in improved methods for detection and control of biological aerosols. This should in turn result in better specification, monitoring and control of bio burden in clean rooms, safer medical facilities and healthier indoor environments. At least two bio burden control technologies are in use currently - EEF and UV technologies. The use of these devices are projected to increase as the large scale commercialization of on line, real time bio burdening monitoring devices occurs.

Literature cited

1. Seaver, M. and J.D. Eversole, "Monitoring Biological Aerosols Using UV Fluorescence", *proc. 15th Annual Meeting AAAR*, p 270, Orlando FL, Oct. 1996.
2. Pinnick, R.G., G. Chen and R.K. Chang, "Aerosol Analyzer for Rapid Measurements of the Fluorescence Species of Airborne Bacteria Excited with a Conditionally Fired Pulsed 266 nm Laser", *proc. 15th Annual Meeting AAAR*, p 271, Orlando FL, Oct. 1996.

Presented at CleanRooms '98 West, San Diego, California. Published in
CleanRooms '98 West Proceedings.

3. Jennings, L.C. and E. C. Dick, "Transmission and Control of Rhinovirus Colds", *European J. of Epidemiology*, Vol 3, No. 4, 327-335, 1987.
4. Jaisinghani, R.A., T.J. Inzana and G. Glindemann, "Biocidal Effects of an Electrically Enhanced Filter", *proc. 15th Annual Meeting AAAR*, p 203, Orlando FL, Oct. 1996.
5. Hoenig, S.A.; Sill, G.T.; Kelley, L.M.; Garvey, K.J.; "Destruction of Bacteria and Toxic Chemicals by a Corona Discharge" *Air Pollution Control Association*, no 3, pg 277-278, March 1980
6. Rhodes, W.W., M.G. Rinaldi and G.W Gorman "Reduction and Growth Inhibition of Microorganisms in Commercial and Institutional Environments", *Env. Health*, 12, Oct. 1995.
7. Tolliver, D.I. "Domestic and International Issues in Contamination Control Technologies", *Microcontamination*, Vol 6, No. 2, 18, Feb. 1988.
8. Jaisinghani, R. A. U.S. patent 543,383 April 4 1995..
9. Jaisinghani, R.A., T.J. Inzana and G. Glindemann, "New Bactericidal Electrically Enhanced Filtration System for Cleanrooms", paper to be presented at the IEST 44th Annual Technical Meeting, Phoenix, AZ, April, 1998.
10. Sarpino, P.V. and N.J. Jensen, "Mechanisms of Removal and Inactivation/Kill of Test Bacteria in Passage Through UV Germicidal Irradiation- Containing Air Cleaning Fixtures", *proc. 15th Annual Meeting AAAR*, p 273, Orlando FL, Oct. 1996.
11. Miller-Leiden, S., M. Hernandez and J.M. Macher, "Effectiveness of Ultraviolet Irradiation of Room Air for Inactivating Airborne Bacteria", *proc. 15th Annual Meeting AAAR*, p 272, Orlando FL, Oct. 1996.
12. Lin, W-H and C-S Li, "The Effect of Sampling Time and Flow Rates on the Bioefficiency of Three Fungal Spore Sampling Methods", *Aerosol Sc. & Tech.*, v. 28, 6., 511, 1998.