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Biological Threat Detection in the Air and on the Surface: How to Define the Risk

Elżbieta Anna Trafny · Rafał Lewandowski · Małgorzata Stępińska · Miron Kaliszewski

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Abstract The improvements in the existing methods of rapid detection and biological pathogen surveillance are still needed. The new spectroscopic methods that rely on the unique structural features and intrinsic fluorescence of microorganisms are well fitted for monitoring the spread of airborne biological agents or their reagentless detection in the air, and these methods may bring a new quality to bioaerosols remote detection. This review describes the problem of the confidence in the environmental testing results that may affect clearance standard, sampling techniques, and the estimation of risk of human exposure to the low concentrations of harmful microorganisms during bioterrorist event or naturally occurring outbreaks. Higher recovery efficiency of dangerous biological agents from the air and contaminated surfaces would enable more reliable environmental human risk exposure assessment.

Keywords Bioterrorism · Bioaerosols · Stand-off detection · Microbial identification

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E. A. Trafny (✉) · R. Lewandowski · M. Stępińska
Department of Microbiology, Military Institute of Hygiene and Epidemiology, Kozielska 4, 01-163 Warsaw, Poland
e-mail: trafny.elzbieta@gmail.com

M. Kaliszewski
Institute of Optoelectronics, Military University of Technology, Warsaw, Poland

Introduction

Rapid and reliable detection of biological agents (microorganisms and toxins) is a key element in proper management of deliberate and/or naturally occurring outbreaks, and especially their consequences. The proper handling of sampling and analysis procedures would minimize contamination of public facilities and infrastructures (food and water supplies) as well as personal exposure.

The bioterrorist event that occurred in autumn 2001 in the United States has serious worldwide implications, received a lot of attention and increased the public perception of risk and danger that might occur in the immediate vicinity (Centers for Disease Control and Prevention 2001; Sanderson et al. 2002). It was also an unexpected event for federal government agencies, which that time had to face the real threat to public health (Ho and Duncan 2005). This threat occurred when letters containing *Bacillus anthracis* endospores ($\sim$1–2 g) were sent through the United States mail system. The letters were received and opened by final recipients and then, the deadly *B. anthracis* endospores were aerosolized and inhaled. This has led to serious infections, which finally caused the death of five persons. The most striking case of exposure to anthrax endospores in fall 2001 was the episode of the retired 94-year-old woman in Connecticut, who received a letter without *B. anthracis* spores inside, but which was previously unfortunately cross-contaminated in the sorting mail facility in Washington, DC (Day 2003).

The anthrax infections in fall 2001 (some cases were of an unknown source) posed once again a question to the public health authorities about the infectious dose of *B. anthracis* endospores for humans. For obvious and legitimate reasons there were not experimental data on the response of humans to bioaerosols containing *B. anthracis*

endospores. The scarce data that are currently available came from analysis of the putative lethal dose of *B. anthracis* endospores in industrial employees working with the products of infected animals (1900's), non-human primate's data, surveys of endemic anthrax in Turkey, human data collected from the Sverdlovsk outbreak in Russia, and recently, from the bioterroristic attack in the USA (Wilkening 2006). The final conclusion from the above considerations is that many previously held assumptions regarding the infectious dose for humans may not be actually correct, and that even one hundred endospores may be lethal for a person with weakened immune response. The recent Exposure–Infection–Symptomatic Illness–Death model published by Toth et al. (2013) showed that a dose of *B. anthracis* endospores leading to infection of 50 % of the exposed persons (ID_{50}) is about 11,000 with a 95 % confidence interval (95 % CI) being between 7,200 and 17,000 endospores. To cause infection in 10 % of susceptible hosts, about 1,700 endospores are required (95 % CI: 1,100–2,600), and, respectively, to induce anthrax infection in one person out of 100 exposed–160 endospores (range 100–250) may be a sufficient dose.

Therefore, an important question arises on how to monitor the environment to ensure the safety of people after the intentional or accidental release of *B. anthracis* endospores and to prevent human exposure to hazardous biological agent?

How Clean is Sufficient?

B. anthracis endospores can survive in the environment for an extremely long time (even a 100 years). The endospores can be re-aerosolized in the air (suspended one more time) and can settle on the surfaces once again (Hong et al. 2010). Therefore, reliable methods for detection of endospores in bioaerosols and on the contaminated surfaces are urgently needed to prevent human cutaneous and inhalational exposure. The most important question is about the limit of detection of the methods being currently in use as well as their reliability and precision (Lim et al. 2005). The endospores can settle on various surfaces, e.g. concrete, carpet, glass, vegetation, and their sampling and analysis should provide a comprehensive answer to a question whether these endospores present on surfaces or in the air could lead to human infection. The main problem in achieving this goal is that most analytical methods cannot detect very low levels of biological contamination. Furthermore, the lack of standardization of analytical methods means that the results are not always reliable and reproducible. For this reason, the current measurements of surface contamination are hardly sufficient for conclusive decisions of public health authorities, such as termination of decontamination process or reopening of the closed public facilities. A cleanup of the facilities contaminated during 2001 anthrax attack lasted at least for 2 years and had costed hundred million dollars (Canter et al. 2005; Schmitt and Zacchia 2012). However, we are still lacking the comprehensive answer to the question under what conditions a significant risk arises from re-aerosolization of *B. anthracis* endospores. We are also not aware whether the standard of zero viable endospores was necessary during remediation of the buildings contaminated in 2001 anthrax attack, and if would be required in the future in case of emergency. It is also necessary to bear in mind that without validated methods of sampling and analysis, the reliability of the negative sampling results might be questioned.

In the recent study of Hong et al. (2010), the link between the environmental concentrations of *B. anthracis* endospores with human risk from exposure to the air-borne biological agent settled on the walls, floors and ventilation system filters was assayed. The results of this study clearly showed that the concentrations of *B. anthracis* endospores that may pose a risk for susceptible person would be extremely low. The concentration of 35 endospores per square meter may already cause a risk (equal to 10^{-3} probability) and to detect such low level of biological contamination one should sample the surface of 485 m^2 with a collection efficiency of 100 %. It is important to mention here that such collection efficiency is not actually achievable. The discussion on the possible collection efficiency using current surface sampling methods is presented below.

Surface Sampling

Environmental sampling for pathogens is a critical component in biological threat detection and in outbreak investigations. Surface sampling for microbiological contaminants is also particularly important in these situations where a high level of environmental hygiene is desired, e.g. in food production, pharmaceutical industry, health care facilities or even spacecraft-related applications. Assessing the surface contamination provides valuable information for epidemiologists regarding the source and potential number of people exposed to an infectious agent. In such situations, one of the purposes of the investigation is post-decontamination sampling to ensure the reduction of risk for public health. The intentional contamination of several US facilities with *B. anthracis* endospores in 2001 has emphasized the importance of standardization and validation of sampling procedures for detecting *B. anthracis* from various types of surfaces (Centers for Disease Control and Prevention 2012; Hodges et al. 2010; Rose et al. 2011).

One of the important issues in methods standardization is their recovery efficiency (RE) that may vary on different environmental surfaces. Some studies on this important

topic have already indicated that a type of sampling device and the structure of contaminated surface may both significantly influence the recovery of *Bacillus* endospores (Rose et al. 2004).

Sample collection devices include dry swabs, premoistened swabs, wipes, high-efficiency particulate air (HEPA) vacuums and aspirating needles (Buttner et al. 2001, 2004a, b; Calfee et al. 2013). The polyurethane-based materials (e.g. macrofoam) have already been identified as a sampling material with high recovery efficiencies (Buttner et al. 2004b; Edmonds et al. 2009; Hodges et al. 2006; Lewandowski et al. 2010). The similar level of contamination of the Brentwood Mail Processing and Distribution Center in Washington, DC with *B. anthracis* endospores was detected with the wipe and HEPA vacuum methods. At the same time the wet-swab and dry-swab methods did not detect over 33 and 66 % endospores present on contaminated surfaces, respectively. No single sampling method was found to collect 100 % of the endospores on the surfaces (Sanderson et al. 2002).

Many of the current protocols for *B. anthracis* endospores detection have been evaluated on stainless steel (Hodges et al. 2006; Lewandowski et al. 2010; Rose et al. 2004), or bare concrete coupons (Brown et al. 2007a, b; Buttner et al. 2004b), and only few investigations have been performed on rough surfaces, e.g. carpets (Buttner et al. 2001; Estill et al. 2009). Recently Krauter et al. (2012) have compared the mean RE values of *B. anthracis* endospores with the roughness index of the surfaces and showed that ceramic tile and stainless steel had the highest mean RE values (48.9 and 48.1 %, respectively). The lowest mean RE (9.8 %) was observed for plastic.

The efficiency of different sampling and sample processing methods was usually assayed in highly sanitized laboratory conditions on clean surfaces (Brown et al. 2007a; Edmonds et al. 2009; Estill et al. 2009; Frawley et al. 2008; Hodges et al. 2006). However, sampling of the surfaces contaminated with *B. anthracis* endospores and other biological (e.g. *Penicillium chrysogenum)* materials proved that in these circumstances the RE values were even higher when compared to the RE value for endospores collected from clean surfaces (Buttner et al. 2001). However, the studies mentioned above were performed in laboratory conditions; therefore, these results need to be confirmed in outdoor tests.

The outdoor tests were also done; some of them were conducted inside the buildings in the environmental conditions that mimic the intentional release of biological agents (Amidan et al. 2007; Piepel et al. 2009). Piepel and coworkers (2012) summarized results from the 20 laboratories and have pointed out that in addition to experimental laboratory tests and indoor tests, the investigations performed under outdoor realistic conditions are needed to reliably establish true RE values of various sampling methods.

After intentional release of biological agents under outdoor urban conditions, the investigation of the contamination level using a wide-area surface sampling, may be complicated by the fact that outdoor urban surfaces are commonly coated with grime layers composed of airborne crustal material, vehicle exhaust, and other urban pollutant aerosols (Lam et al. 2005; Liu et al. 2003). The amount of grime present on the surfaces tested may influence the recovery of endospores with wipes, swabs, and vacuum techniques. Einfeld et al. (2011) assessed the efficiency recovery of the wipe or vacuum method on grime-coated outdoor surfaces and reported that the RE values of these methods are generally higher when grime-coated stainless steel, marble, glass, and concrete surfaces were sampled in comparison to clean surfaces tested under the same conditions. Therefore, the outdoor tests confirmed the results of previous laboratory investigations.

Similar observations have been made in a swab validation study for *B. anthracis* (Hodges et al. 2010). The mean percent RE reported from 12 laboratory response network affiliated laboratories ranged 15.8–31.0 %, when swabbing was performed on surfaces contaminated with *B. anthracis* endospores only. When swabs were used to collect endospores from surfaces already contaminated with background dust or other microorganisms, the RE values were significantly higher (in the range 27.9–55.0 %, respectively). The endospores were collected with premoistened macrofoam swabs in these experiments. However, it is actually unclear how different chemical pollutants may influence the RE value and the numbers of bacteria collected from contaminated surfaces.

The RE values are also affected by technique of sample processing (Downey et al. 2012; Moore and Griffith 2007). In the sample processing protocols, the optimal extraction solution and the method of physical disruption of bacterial aggregates are critical. The techniques of sonication, vortexing, and stomaching are currently the methods most commonly used for these purposes. The period of time for extraction of microorganisms from the complex matrices ranges from 30 s to 15 min, and usually bacteria are extracted to phosphate-buffered saline, water, Butterfield's buffer, or potassium phosphate buffer. In recent years, different surfactants (e.g. Tween 80 or Tween 20) were often added to the extraction solution as wetting agents. They improved release of adherent microorganisms from the contaminated surfaces and reduced losses caused by aggregation of microorganism and their adhesion to walls of tubes during sample processing (Da Silva et al. 2011). Buttner et al. (2001) concluded on the basis of the swab and wipe sampling methods that the majority of the inefficiency was associated with a processing step instead of

the sample collection step. On the contrary, Rose et al. (2004) showed that the REs values for sample collection and processing steps were similar. However, in most of the studies, inefficiency of sample processing is large enough to have a significant impact on the overall RE of a sampling method used.

In the previous experimental studies, the endospore recovery was analyzed usually for single *Bacillus anthracis* strain (e.g. *B. anthracis* Sterne) or individual strains of different *B. anthracis* surrogates (most commonly *B. atrophaeus*) (Edmonds et al. 2009; Hodges et al. 2006). The estimation of the RE values for different surface sampling methods is more difficult when performed simultaneously on both *B. anthracis* endospores and its surrogates than on single species of *Bacillus*, since requires the analysis of many more variables. Probst et al. (2010) have recently reported that a significant difference in successful detection of the two *Bacillus* species exists and result from a two-fold increase of the RE values when *B. atrophaeus* endospores were swabbed from stainless steel coupons over the RE values when of *B. anthracis* Sterne endospores were collected.

The RE value is dependent also on the method used for microorganisms deposition on surfaces. Generally, bacteria in liquid (water and/or ethanol) are used as inocula to contaminate the coupons and are consequently dried after deposition (Frawley et al. 2008; Hodges et al. 2006). However, to best mimic the true situation of microbial surface contamination, one should use the aerosolized microorganisms that would settle down on surfaces. There is a significant difference between the RE values of endospores deposited by these two different methods on surfaces (Edmonds et al. 2009).

The effect of concentration of bacterial inoculum that was seeded on the surface has also a direct correlation to the RE values of sampling materials. In the swab validation study presented by Hodges et al. (2010), coefficients of variation for the RE values ranged 9.4–32.3 %, when a macrofoam swabs were used for sampling. The RE values were also dependent on the inoculum concentration, with greater precision at higher concentrations. Although, many studies were performed in this field so far, additional studies are needed, because our knowledge on the most effective surface sampling methods is still incomplete.

Bioaerosol Detection

Bioaerosols are composed of microorganisms (bacteria, fungi, and algae), biological structures for reproduction and environmental survival, such as pollens, fungal, and bacterial spores as well as fragments of various microorganisms: endotoxins or/and mycotoxins in size ranges of 0.02–100 μm (Després et al. 2012).

There are several methods that may be used as early warning techniques for the detection of dangerous biological agents in the air (Dybwad et al. 2014). Among them the physical methods are the best suited for this purpose, since they provide real-time results, may be operated continuously 24 h per 7 days a week. These methods also require minimal maintenance and no additional laboratory work to produce the consistent results.

Physical Methods of Biological Threat Agents' Detection

Airborne particles like bacteria, fungi, and pollens are complex structures composed of various biomolecules and chemical compounds, which fluoresce (emits visible light when excited with UV) at specific excitations and emission wavelengths (Lakowicz 2006). Two spectral bands of naturally occurring fluorophores are usually considered as those, which allow detection in remote locations and discrimination of bacterial species in the air. The first one is attributed to fluorescent amino acids: tryptophan, tyrosine, and phenylalanine and presents excitation and emission maxima at 280 and 350 nm, respectively. The second band is related to the reduced nicotinamide-adenine dinucleotides (NADH, NADPH) and presents excitation and emission maxima at about 340 and 450 nm, respectively (Hill et al. 2009). Bacterial spores contain fluorescent dipicolinic acid, flavins, and nicotinamide compounds. Currently, the fluorescence spectra could not be used for accurate species determinations: specific identification of microorganisms still have to be performed with immunochemical or molecular techniques. Nevertheless, it has been shown that using intrinsic fluorescence allows efficient remote detection of biological material without any specific preparation of the sample required (Christensen et al. 2006; DeFreez 2009).

The phenomenon of autofluorescence of the biological molecules could be used for detection of microbiological contamination in the air, e.g. for a single particle detection (in situ) in bioaerosols. Based on intrinsic fluorescence of microorganisms, various systems for real time, reagentless, and continuous monitoring of their presence in ambient air have been developed.

In a single particle detection technique, the operation idea is very similar to a flow cytometry. The bioaerosol is drawn through the nozzle and then is aerodynamically focused with filtered, particle free air. The air flux crosses with laser beam, which is scattered on the flowing particles. This phenomenon allows both particles counting and analysis of their size in a real-time schedule. Laser emits energies strong enough to induce fluorescence of fluorophores that are present in single microbial cells. The method, called a laser-induced fluorescence (LIF) allows

differentiation between biological and non-biological particles and classification of microorganisms into groups. In situ detectors developed in several laboratories differ due to number and types of excitation lasers used or number of detected wavelengths (Brosseau et al. 2000; Hairston et al. 1997; Sivaprakasam et al. 2011). Initially these systems were oriented to military applications related to the biological warfare agents' detection. Nowadays, continuous monitoring of ambient air is also of interest for public health authorities with potential applications in hospitals, airports, and for those suffering from allergy (Brosseau et al. 2000; Mitsumoto et al. 2009; Pan et al. 2011).

The intrinsic fluorescence of microorganisms can also be used for stand-off detection of biological cloud. This could be done with second type of biodetectors, i.e. a light detection and ranging (LIDAR) system. The devices allow the bioaerosol cloud detection and classification of microorganisms into groups in open area at distances up to several kilometers away. The laser beam reaching bioaerosol cloud is scattered and detected back via telescope. The scattering signal allows determination of the distance and depth of the cloud. If the cloud is of biological origin, it always induces fluorescence signal that can be detected and registered (Farsund et al. 2012; Joshi et al. 2013; Mierczyk et al. 2011).

The stand-off detection is recognized as early warning system (in the Detect-to-Protect window) against bioterrorist attack. The great advantage of a LIDAR is possibility of vertical and horizontal scanning of a very large area. It allows calculation and visualization of dimensions, distance, and movement of the cloud. The two LIDAR systems for real-time stand-off detection of bioagents have recently been developed in Poland and resulted from the scientific projects supervised by the Institute of Optoelectronics at Military University of Technology in Warsaw. Both systems use the intrinsic fluorescence phenomena for differentiation between biological and non-biological aerosols. The first, short-range system is designed for bioaerosol detection at distances up to 200 m. It is based on one 375 nm CW laser, a receiving telescope and three detection channels. The scattering signal recorded in the first channel is used for bioaerosol detection. Differentiation between biological and non-biological particles is realized with two fluorescence channels. In the second mid-range LIDAR system, the detection is based on two independent physical phenomena: a LIF and depolarization that results from elastic scattering on non-spherical particles. This device includes three laser sources, two receiving telescopes, depolarization component, and a spectral signature analyzing spectrograph. It was designed to provide the stand-off detection capability at ranges from 200 m up to several kilometers. The system is on a mobile platform for vehicle or building installation. Additionally, the system is combined with a scanning mechanics and advanced software, which enable to conduct the semi-automatic monitoring of a specified space sector (Mierczyk et al. 2011). Both systems were successfully tested during EURO 2012 competition in Warsaw and also at the Dugway Proving Ground in 2013 in Utah (USA).

The spectroscopic methods based on intrinsic fluorescence of microorganisms can also be applied for a direct and reagentless detection of microorganisms on a surface. The successful trials on using above phenomenon to measure the microbial meat spoilage with UV excitation of wavelengths longer than 280 nm have already been identified (Aït-Kaddour et al. 2011; Sahar et al. 2011). It is worth to mention that differentiation between protein originated from meat and the bacteria was possible due to strong signal of microorganisms resulted from their number. Only few publications have shown the possibility of detecting small microbial clusters on natural surfaces because most solid surfaces show high fluorescence levels under UV light (280–380 nm). During the last few years, a new and relatively compact laser sources have appeared on the market. They emit wavelengths shorter than 250 nm (deep UV). Recently, Bhartia et al. (2010) reported that visualization of marine microorganisms on a stone can be achieved with the use of 224 nm laser. The possibility of using deep UV range for improved detection and classification of biological agents was also reported by our group (Włodarski et al. 2010). Currently, deep UV fluorescence microscopic system allowing visualization of microorganisms directly on surfaces like stones, ceramics, bricks, furniture, cellulose filters, etc. is being developed in the Institute of Optoelectronics at Military University of Technology in Warsaw.

All fluorescence spectra obtained from different microbial species share protein and NADH bands. They differ regarding to the intensity ratio between bands and/or the shape. However, the analysis and comparison of huge number of data is difficult, since bioaerosols could be characterized by various physical parameters like fluorescence spectra from one or more excitation wavelengths, scattering, polarization, and particle size (Agranovski et al. 2003; Huang et al. 2008; Pan et al. 2003, 2011).

The huge amount of information collected when physical methods are being used for measurements needs both the algorithms of data selection and the advanced statistical methods to be efficiently processed. Principal component analysis and hierarchical cluster analysis methods have been successfully applied for the analysis of spectroscopic data and classification of bioaerosols. Both statistical methods allow graphical representation of data, and huge number of recorded parameters of the sample can be reduced to a single point. In general, the samples

representing similar properties would be placed in close vicinity as result of these statistical analyses (Bombalska et al. 2010; Cebredo et al. 2007; Kaliszewski et al. 2013; Mularczyk-Oliwa et al. 2012).

Sampling and Analysis of Bioaerosols Using Microbiology and Molecular Biology Techniques

Generally, there are three groups of methods that may be used to collect a bioaerosol sample: impaction of biological particles (on microbiological solid media), filtration (capture on filters, e.g. gelatine filters that also concentrate viral particles) and impingement (e.g. in impingers or cyclones) that collect microorganisms into the liquid (Xu et al. 2011). These methods have some advantages and disadvantages; though, one of the most important issues during bioaerosol sampling is to preserve the viability of microorganisms, since viable agents might be further tested for their infectivity, pathogenicity, and virulence.

The molecular methods are most useful in determination of characteristic features (identification) and detection of virulent traits in genomes of microorganisms. Among them, polymerase chain reaction (PCR) and quantitative real-time PCR (RT-PCR) have recently emerged as leading technologies, since they are rapid, sensitive, and permit a multiplexed pathogen detection and characterization (Kim et al. 2011). However, RT-PCR has several disadvantages, as follows: high cost of equipment, the substantial technical expertise required and high degree of sensitivity and specificity that may lead to false positives or negatives. At the same time, quantitative RT-PCR has also an important positive feature, since it allows detecting and enumerating the viable but nonculturable bacteria (VBNC), thereby providing the solution to the common problem of bioaerosols analysis, i.e. loss of viability of microorganisms due to aerosol sampling. The VBNC bacterial cells have a reduced ability to grow on microbiological solid media (cultivability) (Kaushik and Balasubramanian 2012).

Several other molecular methods are also currently developed to allow rapid analysis of bioaerosol samples, i.e. high-density protein arrays for the microorganisms, high-throughput sequencing, fluorescent staining methods (e.g. fluorescence in situ hybridization) (Irenge and Gala 2012). Flow cytometry has also been successfully applied in analysis of air samples (Chen and Li 2007).

Despite the very rapid development of molecular techniques, the methods of classical microbiology, i.e. culture and enumeration of microorganisms on solid media, have not lost their importance and are still used in a large number of research centers. It should also be noted that during the 2001 anthrax attack, the traditional culture showed a higher sensitivity for the detection of the samples contaminated with *B. anthracis* spores than the molecular methods (Higgins et al. 2003). In the recent study, Hospodsky et al. (2010) have shown that overall method detection limit for a quantitative RT-PCR was in the range 2,000–3,000 spores of *Bacillus atrophaeus*, a well-known surrogate for *B. anthracis*. The extraction efficiency of the spores from filters, as well as the extraction efficacies of DNA from the spores were both taken into account in these calculations. Other factors that might influence the detection limit of the molecular techniques, when analyzing the samples of bioaerosols, were the collection efficiencies of sampling instruments (which are actually not well known for many samplers at low template concentrations) and inhibition of the polymerase activity that may come from the complex sample matrices.

The collection efficiency of two impactors: Andersen six-stage impactor and AirPort MD8 with gelatin filters were compared with the efficiency of two all glass impingers. The highest physical efficiency was shown for AirPort MD8 and it was five times higher that for Andersen six-stage impactor (18 %) (Zhao et al. 2011). For two all glass impingers, the intermediate values of collection efficiency were demonstrated.

When microbiological culture methods were used for bacterial enumeration at low target concentrations (10^2–10^4 CFU/m^3), the lowest concentration of endospores that may be reliable sampled with impactor AirPort MD8 was shown to be in the range 1,000–1,500 CFU/m^3. These values resulted from the experiments performed in the chambers with *B. atrophaeus* or *B. anthracis* Sterne endospores aerosolized in both HEPA-filtered and ambient room air (Estill et al. 2011; Lewandowski et al. 2013). The use of a pour-plate method instead of a plate-spread method even without additional pre-concentration step improved the limit of endospores detection in these experiments.

The lowest volume of the liquid, to which biological particles are impinged, is 5 ml in the commercially available sampling devices (impingers and cyclones). Therefore, when one species of microorganisms is targeted, e.g. *B. anthracis* spores at a relatively low concentration in ambient air, it seems reasonable to introduce an additional concentration/separation step during the bioaerosol analysis, for example, an immunomagnetic separation technique. This method uses paramagnetic beads coated with antibodies of high affinity to target organisms that capture the bacteria (endospores) with high binding efficiency, thereby concentrating and purifying the target organisms in suspensions. The immunoseparation technique has been widely used to separate the target organisms from complex matrices, such as food, soil, or water. When used for secondary concentration of bioaerosol sample, this method may improve the detection limits (Fisher et al. 2009) of both microbiological and molecular methods.

The observations above underline the difficulty in reliable measurements of low concentrations of dangerous biological agents in air. It has been shown that at least seven successful detection events out of seven trials are needed to detect five spores from a reference *B. atrophaeus* sample with 99 % confidence (Hospodsky et al. 2010). Therefore, a zero risk for humans cannot be easily established, since without many repetitions the variability of sampling and analysis may lead to negative result (Hong et al. 2010).

Conclusions

The anthrax attack in 2001 caused a return to the basic issues of environmental hygiene and put on new questions about the reliability of the methods used for years in the research from this field. Current observations show that we still need reliable and not expensive methods for sampling and analysis of environment where there is a significant risk for human health. We still are lacking the answers for the following questions: (1) what is a clearance standard (such as "zero tolerance") in outdoor areas and wide areas; (2) what technologies for sampling the surfaces and air have already been validated; (3) how to recover *B. anthracis* spores from complex material; and (4) what are the best methods of decontamination that do not influence the sampling efficiency. Getting answer to these questions would increase confidence in the results of environmental testing and decrease the risk of exposure to harmful environmental factors.

References

Agranovski V, Ristovski Z, Hargreaves M et al (2003) Real-time measurement of bacterial aerosols with UVAPS: performance evaluation. J Aerosol Sci 34:301–317

Aït-Kaddour A, Boubellouta T, Chevallier I (2011) Development of a portable spectrofluorimeter for measuring the microbial spoilage of minced beef. Meat Sci 88:675–681

Amidan BG, Piepel GF, Matzke BD et al (2007) Experimental design for the INL sample collection operational test, PNNL-17129. Pacific Northwest National Laboratory, Richland, WA. Available at: http://www.pnnl.gov/main/publications/external/technical_reports/PNNL-17129.pdf. Accessed 19 Feb 2014

Bhartia R, Salas EC, Hug WF et al (2010) Label-free bacterial imaging with deep-UV-laser-induced native fluorescence. Appl Environ Microbiol 76:7231–7237

Bombalska A, Mularczyk-Oliwa M, Kwaśny M et al (2010) Classification of the biological material with use of FTIR spectroscopy and statistical analysis. Spectrochim Acta A Mol Biomol Spectrosc 78:1221–1226

Brosseau LM, Vesley D, Rice N et al (2000) Differences in detected fluorescence among several bacterial species measured with a direct-reading particle sizer and fluorescence detector. Aerosol Sci Tech 32:545–558

Brown GS, Betty RG, Brockmann JE et al (2007a) Evaluation of vacuum filter sock surface sample collection method for *Bacillus* spores from porous and non-porous surfaces. J Environ Monit 9:666–671

Brown GS, Betty RG, Brockmann JE et al (2007b) Evaluation of rayon swab surface sample collection method for *Bacillus* spores from nonporous surfaces. J Appl Microbiol 103:1074–1080

Buttner MP, Cruz-Perez P, Stetzenbach LD (2001) Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. Appl Environ Microbiol 67:2564–2570

Buttner MP, Cruz-Perez P, Stetzenbach LB et al (2004a) Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis. Appl Environ Microbiol 70:4740–4747

Buttner MP, Stetzenbach LD, Klima-Comba AK et al (2004b) Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. Appl Environ Microbiol 70:7040–7045

Calfee MW, Rose LJ, Morse S et al (2013) Comparative evaluation of vacuum-based surface sampling methods for collection of *Bacillus* spores. J Microbiol Methods 95:389–396

Canter DA, Gunning D, Rodgers P et al (2005) Remediation of *Bacillus anthracis* contamination in the US Department of Justice mail facility. Biosecur Bioterror 3:119–127

Cebredo S, Parra A, Anzano J (2007) Bacteria spectra obtained by laser induced fluorescence. J Fluoresc 17:171–180

Centers for Disease Control and Prevention (2001) Evaluation of *Bacillus anthracis* contamination inside the Brentwood mail processing and distribution center B—District of Columbia. Morb Mortal Wkly Rep 50:1129–1133

Centers for Disease Control and Prevention (2012) Surface sampling procedures for *Bacillus anthracis* spores from smooth, nonporous surfaces. Available at: http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html Accessed 27 Feb 2014

Chen PS, Li CS (2007) Real-time monitoring for bioaerosols—flow cytometry. Analyst 132:14–16

Christensen J, Nørgaard L, Bro R et al (2006) Multivariate autofluorescence of intact food systems. Chem Rev 106:1979–1994

Da Silva SM, Filliben JJ, Morrow JB (2011) Parameters affecting spore recovery from wipes used in biological surface sampling. Appl Environ Microbiol 77:2374–2380

Day GD (2003) The autumn 2001 anthrax attack on the United States postal service: the consequences and response. J Conting Crisis Manag 11:110–117

DeFreez R (2009) LIF bio-aerosol threat triggers: then and now. Optically Based Biological and Chemical Detection for Defence V. Proc SPIE, 7484:74840H

Després V, Huffman J, Burrows S et al (2012) Primary biological aerosol particles in the atmosphere: a review. Tellus B, North America, 64. Available at: http://www.tellusb.net/index.php/tellusb/article/view/15598. Accessed 27 Feb 2014

Downey AS, Da Silva SM, Olson ND et al (2012) Surface sampling of bacteria from wipes used in biological impact of processing method on recovery. Appl Environ Microbiol 78:5872–5881

Dybwad M, Skogan G, Blatny JM (2014) Temporal variability of the bioaerosol background at a subway station: concentration level, size distribution, and diversity of airborne bacteria. Appl Environ Microbiol 80:257–270

Edmonds JM, Collet PJ, Valdes ER et al (2009) Surface sampling of spores in dry-deposition aerosols. Appl Environ Microbiol 75:39–44

Einfeld W, Boucher RM, Tezak MS et al (2011) Evaluation of surface sampling method performance for *Bacillus* spores on clean and dirty outdoor surfaces, SAND2011-4085. Sandia National Laboratories, Albuquerque

Estill CF, Baron PA, Beard JK et al (2009) Recovery efficiency and limit of detection of aerosolized *Bacillus anthracis* Sterne from environmental surface samples. Appl Environ Microbiol 75:4297–4306

Estill CF, Baron PA, Beard JK et al (2011) Comparison of air sampling methods for aerosolized spores of *B. anthracis* Sterne. J Occup Environ Hyg 8:179–186

Farsund Ø, Rustad G, Skogan G (2012) Standoff detection of biological agents using laser induced fluorescence—a comparison of 294 nm and 355 nm excitation wavelengths. Biomed Opt Express 3:2964–2975

Fisher M, Atiya-Nasagi Y, Simon I et al (2009) A combined immunomagnetic separation and lateral flow method for a sensitive on-site detection of *Bacillus anthracis* spores—assessment in water and dairy products. Lett Appl Microbiol 48:413–418

Frawley DA, Samaan MN, Bull RL et al (2008) Recovery efficiencies of anthrax spores and ricin from nonporous or nonabsorbent and porous or absorbent surfaces by a variety of sampling methods. J Forensic Sci 53:1102–1107

Hairston PP, Ho J, Quant FR (1997) Design of an instrument for real-time detection of bioaerosols using simultaneous measurement of particle aerodynamic size and intrinsic fluorescence. J Aerosol Sci 28:471–482

Higgins JA, Cooper M, Schroeder-Tucker L et al (2003) A field investigation of *Bacillus anthracis* contamination of US Department of Agriculture and other Washington, DC, buildings during the anthrax attack of October 2001. Appl Environ Microbiol 69:593–599

Hill SC, Mayo MW, Chang RC (2009) Fluorescence of bacteria, pollens and naturally occurring airborne particles: excitation/emission spectra. Army Research Laboratory ARL-TR-4722

Ho J, Duncan S (2005) Estimating aerosol hazards from an anthrax letter. J Aerosol Sci 36:701–719

Hodges LR, Rose LJ, Peterson A et al (2006) Evaluation of a macrofoam swab protocol for the recovery of *Bacillus anthracis* spores from a steel surface. Appl Environ Microbiol 72:4429–4430

Hodges LR, Rose LJ, O'Connell H et al (2010) National validation study of a swab protocol for the recovery of *Bacillus anthracis* spores from surfaces. J Microbiol Methods 81:141–146

Hong T, Gurian PL, Ward NF (2010) Setting risk-informed environmental standards for *Bacillus anthracis* spores. Risk Anal 30:1602–1622

Hospodsky D, Yamamoto N, Peccia J (2010) Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi. Appl Environ Microbiol 76:7004–7012

Huang HC, Pan Y, Hill SC et al (2008) Real-time measurement of dual-wavelength laser-induced fluorescence spectra of individual aerosol particles. Opt Express 16:16523–16528

Irenge LM, Gala JL (2012) Rapid detection methods for *Bacillus anthracis* in environmental samples: a review. Appl Microbiol Biotechnol 93:1411–1422

Joshi D, Kumar D, Maini AK et al (2013) Detection of biological warfare agents using ultra violet-laser induced fluorescence LIDAR. Spectrochim Acta A Mol Biomol Spectrosc 112:446–456

Kaliszewski M, Trafny EA, Lewandowski R et al (2013) A new approach to UVAPS data analysis towards detection of biological aerosol. J Aerosol Sci 58:148–157

Kaushik R, Balasubramanian R (2012) Assessment of bacterial pathogens in fresh rainwater and airborne particulate matter using real-time PCR. Atmos Environ 46:131–139

Kim SY, Kim ZY, Lee S et al (2011) Comparison of molecular and total ATP-based analytical methods with culture for the analysis of bioaerosols. Sci Total Environ 409:1732–1737

Krauter PA, Piepel GF, Boucher R et al (2012) False negative rate and other performance measures of a sponge-wipe surface sampling method for low contaminant concentrations. Appl Environ Microbiol 78:846–854

Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Springer, New York

Lam B, Diamond ML, Simpson AJ et al (2005) Chemical composition of surface films on glass windows and implications for atmospheric chemistry. Atmos Environ 39:6578–6586

Lewandowski R, Kozłowska K, Szpakowska M et al (2010) Use of a foam spatula for sampling surfaces after bioaerosol deposition. Appl Environ Microbiol 76:688–694

Lewandowski R, Kozłowska K, Szpakowska M et al (2013) Evaluation of applicability of the Sartorius Airport MD8 sampler for detection of *Bacillus* endospores in indoor air. Environ Monit Assess 185:3517–3526

Lim DV, Simpson JM, Kearns EA et al (2005) Current and developing technologies for monitoring agents of bioterrorism and biowarfare. Clin Microbiol Rev 18:583–607

Liu Q, Chen R, McCarry BE et al (2003) Characterization of polar organic compounds in the organic film on indoor and outdoor glass windows. Environ Sci Technol 37:2340–2349

Mierczyk Z, Kopczyński K, Zygmunt M et al (2011) Fluorescence/depolarization lidar for mid-range stand-off detection of biological agents. SPIE Defense Security and Sensing, Orlando

Mitsumoto K, Yabusaki K, Aoyagi H (2009) Classification of pollen species using autofluorescence image analysis. J Biosci Bioeng 107:90–94

Moore G, Griffith C (2007) Problems associated with traditional hygiene swabbing: the need for in-house standardization. J Appl Microbiol 103:1090–1103

Mularczyk-Oliwa M, Bombalska A, Kaliszewski M et al (2012) Comparison of fluorescence spectroscopy and FTIR in differentiation of plant pollens. Spectrochim Acta A Mol Biomol Spectrosc 97:246–254

Pan YL, Aptowicz KB, Chang RK et al (2003) Characterizing and monitoring respiratory aerosols by light scattering. Opt Lett 28:589–591

Pan YL, Hill SC, Pinnick RG et al (2011) Dual-excitation-wavelength fluorescence spectra and elastic scattering for differentiation of single airborne pollen and fungal particles. Atmos Environ 45:1555–1563

Piepel GF, Amidan BG, Matzke BD (2009) Experimental and sampling design for the INL-2 sample collection operational test, PNNL-18187. Pacific Northwest National Laboratory, Richland, WA. Available at: http://www.pnnl.gov/main/publications/external/technical_reports/PNNL-18187.pdf. Accessed 27 Feb 2014

Piepel GF, Amidan BG, Hu R (2012) Laboratory studies on surface sampling of *Bacillus anthracis* contamination: summary, gaps and recommendations. J Appl Microbiol 113:1287–1304

Probst A, Facius R, Wirth R et al (2010) Validation of a nylon flocked-swab protocol for efficient recovery of bacterial spores from smooth and rough surfaces. Appl Environ Microbiol 76:5148–5158

Rose LJ, Jensen B, Peterson A et al (2004) Swab materials and *Bacillus anthracis* spore recovery from nonporous surfaces. Emerg Infect Dis 10:1023–1029

Rose LJ, Hodges L, O'Connell H et al (2011) National validation study of a cellulose sponge wipe-processing method for use after sampling *Bacillus anthracis* spores from surfaces. Appl Environ Microbiol 77:8355–8359

Sahar A, Boubellouta T, Dufour É (2011) Synchronous front-face fluorescence spectroscopy as a promising tool for the rapid determination of spoilage bacteria on chicken breast fillet. Food Res Int 44:471–480

Sanderson WT, Hein MJ, Taylor L et al (2002) Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg Infect Dis 8:1145–1151

Schmitt K, Zacchia NA (2012) Total decontamination cost of the anthrax letter attacks. Biosecur Bioterror 10:98–107

Sivaprakasam V, Lin HB, Huston AL et al (2011) Spectral characterization of biological aerosol particles using two-wavelength excited laser-induced fluorescence and elastic scattering measurements. Opt Express 19:6191–6208

Toth DJ, Gundlapalli AV, Schell WA et al (2013) Quantitative models of the dose-response and time course of inhalational anthrax in humans. PLoS Pathog 9:e1003555

Wilkening DA (2006) Sverdlovsk revisited: modeling human inhalation anthrax. Proc Natl Acad Sci USA 103:7589–7594

Włodarski M, Kaliszewski M, Mularczyk-Oliwa M et al (2010) Comparison of fluorescence of biological materials with deep UV excitation (225 nm, 266 nm and 280 nm). 10th International Symposium on Protection Against Chemical and Biological Warfare Agents, Stockholm, Sweden

Xu Z, Wu Y, Shen F et al (2011) Bioaerosol science, technology, and engineering: past, present, and future. Aerosol Sci Technol 45:1337–1349

Zhao Y, Aarnink AJ, Doornenbal P et al (2011) Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. II: sampling efficiency and half-life time. Aerosol Sci Technol 45:432–442