Use of Virtual Impactor (VI) Technology in Biological Aerosol Detection†

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Abstract

Detecting biological threat aerosol is difficult in that a small cloud lasting only a few seconds at a point location may contain sufficient material to infect large numbers of exposed individuals. Clinical analytical methods require relative large amounts of the sample in liquid form to facilitate positive measurements. Biological agents may be fragile because of their lipid membranes that can be susceptible to harsh sample collection treatment. Damaged organisms may render subsequent analyses to be invalid. Virtual impaction (VI) sample collectors have been theorized to provide usable concentration rates yet are sufficiently gentle with the aerosol particles to preserve cellular viability. This review will discuss different implementations of VI technology and examine their merits. Outstanding issues will be outlined to aid future experimentation.

Keywords: virtual impactor, biological aerosol, anthrax, aerosol samplers, threat agents

Introduction

That humans get infected by microorganisms, mostly from infectious agents transported in the air, is not surprising as people get sick all the time (Verreault et al. 2008). Daily biological threats are such common occurrences that we have become accepting of the fact. Occasionally, concern may be elevated when travellers get infected in enclosed environments like aircraft. Sometimes fatalities do take place, for example, infections caused during hospitalization. Yet, the concern for these episodes has not heightened public awareness to the point where drastic solutions are called for.

However, recent events (Riedel, 2004) have changed that complacency somewhat. What happened in the Middle East in the early 1990’s and subsequent World Trade Center events prompted military and public health organisations to seek solutions that can be used to measure the occurrence of nuclear biological and chemical (NBC) threats. For example, Ho and Duncan (2005) described the anthrax scenario that resulted in two fatalities at the Brentwood postal station in Washington DC. Using knowledge of the aerosol source and location of the targets, they were able to estimate the lethal dosage that caused the fatalities, the first time that this could be done. Blatny et al. (2010) led a team to investigate the spread of airborne Legionella bacteria from a pulp waste treatment plant in Norway that had previously caused a number of fatalities in a nearby town. They discovered that the source of live organisms came from large bubbling tanks that continuously emit aerosol particles into the air. To be clear, prior to this time period, there had always been a low level requirement for ways to detect military threats. But since then, the demand for detection technologies jumped by orders of magnitude and significant funding became available. However, biological detection for threats in militarily or civilians settings have always been a difficult problem to solve. Indeed, for the past thirty years, scientists and engineers from around the world have been engaged in solving the problem. But the issues are so recalcitrant that after billions of dollars spent, the illusive hand held biological detector is still nowhere in sight.

Briefly, the problem can be summarized thus. There is a need to detect, within seconds, a small cloud of particulate biological aerosol being transported by wind over a long distance. The particles

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are superimposed over a background of environmental contaminants. The detection technology has to sort these materials in real time without incurring more than one false alarm per week. Given these requirements, it can be seen how the first requirement for a detector system is to concentrate the particles, preferably while they are still in the air, before submitting them for analysis. For the concentration step, the virtual impactor (VI) appears to be the ideal solution. This review will attempt to focus on the role played by VI technologies in solving problems associated with biological detection. It is assumed that the reader will have familiarity with VI as previously reviewed by Marple (2004). Having an understanding of naturally occurring bioaerosols and their transport characteristics in nature as reviewed by Jones and Harrison (2004) is helpful. Some authors like Eubanks et al. (2007) discussed both chemical and biological threats but this review will be restricted to addressing bacterial and viral threats.

The dichotomous sampler

Although Marple (1970) provided a detailed description of the VI in his PhD thesis, it was Loo and Cork (1988) who designed the dichotomous sampler (DS) using VI technology. The sampler was subsequently marketed by a company called Sierra-Andersen for particle measurements in the environment (Environmental Monitoring Systems, Laboratory, 1985). The usefulness of the DS in characterizing biological aerosol was first demonstrated in outdoor field trials. Ho et al. (1990) used a DS to characterize artificially generated biological aerosol whilst detecting light scatter signals from a standoff laser based system. Further chamber work with the DS (Ho, 1991) led to the discovery that over 80-90% of the live individuals from artificially generated biological aerosol particles were to be found in the >2.5 µm fraction as aggregates. This and a series of related laser standoff biological detection studies were summarized by Evans et al. (1994).

The DS, using one single nozzle coupling, serves as an ideal device to provide a simplified illustration for how VI works (Fig. 1). The Sierra Andersen instrument was designed with an inlet flow rate of 1 m³/hr or 16.7 l/min, shown at the top of the right hand illustration. Particles are focused into a narrow accelerating stream exiting at the “virtual impactor nozzle”. On exit, the bulk of the total flow is split into the “fine” stream (<2.5 µm, 15 l/min) while 1.67 l/m is captured by the “virtual impactor receiver. Particles were collected on glass fiber filters for later analysis (Lai and Chen, 2000). Information on the instrument can be found in the Sierra Andersen instruction manual (Environmental Monitoring Systems, Laboratory, 1985). Mathematical treatment for the VI can be found in Marple and Chien (1980).

Fig. 1  The Andersen model 244 dichotomous sampler (left) and the virtual impactor (right) as implemented in the model 244 and marketed by Sierra Andersen company. The instrument was designed with an inlet flow rate of 1 m³/hr or 16.76 l/min; 15 l/min; 1.67 l/min. Of this total flow, 15 l/min was diverted into the “fine” (<2.5 µm) stream while 1.67 l/min went straight down the “coarse” (>2.5 µm) stream. Particles were collected on glass fiber filters for later analysis (Lai and Chen, 2000). Information on the instrument can be found in the Sierra Andersen instruction manual (Environmental Monitoring Systems, Laboratory, 1985). Mathematical treatment for the VI can be found in Marple and Chien (1980).
tube” that goes straight down the “coarse” (>2.5 µm) stream. The two divergent size segregated particle trains are subsequently collected on separate glass fiber filters for later analysis (Lai and Chen, 2000).

In this simplified illustrated version of VI, it can be seen that the bulk of the particles >2.5 µm are segregated and thus concentrated, a beneficial outcome if the agents of interest are mostly in this size group. In actual implementation of the multi-jet VI technology (Marple and Chien, 1980), the fine particle stream is “dumped” as exhaust. Some workers may be interested in capturing fine particles (<2.5 µm) of a smaller size cutoff. Sioutas et al. (1994) developed a slit version of VI that has a lower particle size 50% cutoff at 0.1-0.25 µm. Conceptually, a lower cutoff size may be of relevance for individual viruses that may appear under electron microscopic view to fit into nanometer size range. However, a low size cutoff is not a significant concern for capturing human transmitted viral aerosol as Tellier (2006) pointed out that influenza virus exist as aggregates of about 5-10 µm in diameter. Recently, Lindsley et al. (2010), measuring particles from human cough, confirmed that 35% of the influenza RNA was contained in particles >4 µm in aerodynamic diameter, while 23% was in particles 1 to 4 µm and 42% in particles <1 µm. The evidence suggests that VI technology may be suitable for collecting bacterial and viral aerosols that exist in the environment.

Early attempts at integrating VI sampling to liquid chemistry

In the 1970s the US Army contracted Bendix Corp. to build the XM19 biological detector (Fig. 2 and 3). It was accompanied by the XM2 liquid collector for saving material for post analysis. Each unit consisted of a virtual impactor that ran at a flow rate of 1200 to 1400 l/min. In the XM19, the large particle fraction was impacted on a moving plastic tape dispensed from a cassette (Barrett and Miller, 1975). Particulate material on the tape was treated with Luminol reagents and if the sampled area contained bound iron in captured bacterial agents (Neufeld et al., 1965), the resultant chemiluminescent signal would be detected via analogue electronics that led to the sounding of an alarm. Simultaneously, the XM2 would be triggered to collect a liquid sample of impinged particles. The liquid sample was to be sent to a microbiological facility for confirmatory studies. Theoretically, the detection approach was based on good microbiological principles (Sotnikov, 1970; Miller and Vogelhut, 1978 and Andre et al., 2003). However, it was not realized at the time, environmental aerosol contained an abundance of non-specific particles rich in iron which caused interference that led to many false alarms (Vong et al. 2007).

Although the XM19 was rejected by the US Army in early 1983 (Smart, 2005), the XM2 was later given a new coat of desert colour paint for deployment during the first Gulf war. In a declassified report (Rostker, 2000), it was mentioned that the collector was used to obtain liquid samples for anthrax testing by handheld test strip technology. It was also mentioned that the test kit was not very effective due to too many false positives.

The mass spectrometry phase

During the mid to late 1990’s, there was a heightened awareness of potential bioterrorism occurrence world wide. Great efforts were put towards chemical
and biological (CB) detection using the most sophisticated technologies regardless of cost. Bruker was a prominent instrument maker associated with mass spectrometry and they were contracted to produce an instrument that could detect CB agents in real time (Griest et al. 2001). To accomplish the required sensitivity and speed, VI technology was implemented (Fig. 4). The desired performance characteristics were ambitious, as seen in this quote:

“The air sampler concentrates size-classified airborne particles that may host toxins, viruses, and other dangerous biological warfare agents such as anthrax spores. These particles are heated in a pyrolyzer in the presence of a methylating reagent. As a result, the membrane lipid fatty acids in the microorganisms break off of the phospholipid and form volatile methyl esters that are ionized in the presence of a chemical ionization reagent gas that converts them into intact charged molecules, or ions. The ions are separated in an ion trap mass spectrometer. A computer examines the resulting mass spectrum and determines whether a biological warfare agent is present in the air; if so, it identifies the agent from its spectrum. For example, bacteria are identified from the presence or absence and ratios of several key fatty acids”. (www.ornl.gov/info/ornlreview/measure/analy/direct/chem-bio.htm [accessed on 9 March 2011]). Fox (2005) provided a detailed account of the development of the US military CBMS system and commented on its susceptibility to false alarms. History has shown that mass spectrometry did not become commonly adopted for general applications in biological detection. However, Bruker took all the lessons learnt from this work and in 2010, marketed an instrument specifically for laboratory identification of clinically relevant microorganisms (Prod’hom et al. 2010). Success in applying such instrument technique required very precise sample preparation as described by Freiwald and Sauer (2009), a regime unlikely to be met by field portable instruments.

An attempt to develop a liquid reagent based biological chemical detector (BCD)

Only a relatively few cells are known to constitute an infectious dose ($10^1$-$10^4$ cells). Borthwick et al. (2005) explained the difficulty in using antibody based immunological methods for measuring infectious dose of a variety of bacterial pathogens. The solution they selected was to sonicate the bacterial spore samples in order to detect $10^6$ spores/ml. Without such treatment, the concentrations required were 10-100 times higher. In the early 1990’s there was a concerted effort to develop a liquid reagent based biological chemical detector (BCD) by automating antibody based detection methods. However, obtaining sufficient biological aerosol material within a short time was a significant hurdle. To develop the BCD, it was thought that VI technology would provide the necessary concentrating effect to gather enough aerosol particles in liquid fractions. Fig. 5a shows a VI designed to concentrate 100 l air and render the particles into a liquid stream at a continuous flow rate of 100 µl/min.

Fig. 4 A portable mass spectrometer coupled to a virtual impactor for concentrating biological aerosol particles (www. armedforces-int.com/article/bruker-daltonics-inc-nbc-instruments.html accessed 9 March 2011).
Several defense laboratories were tasked to characterize the inlet concentrator using a non virulent stimulant for anthrax. Chamber trials using *Bacillus subtilis* (BG) spore aerosol, a method pioneered by Ferry et al. (1949), revealed that on each test occasion, the concentrator had a collection efficiency of 0.01 to 1% (unpublished). When the internal wall of the VI was examined, it was discovered that much of the aerosol particles adhered to the walls at the two orifice stages (Fig. 5c and d). In hindsight, it is now well understood that biological cells naturally adhere to surfaces. Abu-Lail and Camesano (2003) studied the role of polysaccharides in bacterial adhesion using atomic force microscopy (AFM). Using data from a variety of sources, table 1 summarizes the typical adhesion forces measured by AFM. Bakker et al. (2004) observed that different organisms taken from a variety of habitats exhibited different adhesion forces, suggesting that more than just simple physical forces exert an influence. The lesson from this illustration is that in designing VI devices of high throughput efficiency, knowledge of the nature of biological stickiness is essential. Overcoming the problem can be a difficult task. Ironically, the lack of success in making the BCD perform to expectations was not due to low VI concentration efficiency. It was discovered that antibody fractions raised for a strain of anthrax were non specific; there was cross reactivity with other organisms (Phillips and Martin, 1988; Quinlan and Foegeding, 1997; Longchamp and Leighton, 1999). The project was abandoned due to this lack of reaction specificity.

![Fig. 5](image)

**Fig. 5** Virtual impactor designed for a biological detector based on immunological measurements using specific antibody preparations. a. external view showing an attachment to facilitate flow rate measurements, b anemometer probe above the first concentrator stage, c view of first concentrator surface after processing a biological aerosol and d similar view of the second concentrator stage. Part of the surfaces in c & d were wiped clean to provide refractive contrast. Note significant built up of particulate dust on the internal surfaces.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Force (nN)</th>
<th>Level</th>
</tr>
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<tr>
<td>Antibody to E. coli</td>
<td>Glass</td>
<td>0.6</td>
<td>Low</td>
</tr>
<tr>
<td><em>Azospirillum brasilense</em></td>
<td>Extracellular polymers</td>
<td>0.8 ± 0.2</td>
<td>Low</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>Aluminum</td>
<td>5.6 ± 0.8</td>
<td>Medium</td>
</tr>
<tr>
<td><em>Desulfovibrio desulfuricans</em></td>
<td>Aluminum</td>
<td>5.4 ± 0.6</td>
<td>Medium</td>
</tr>
<tr>
<td>Bacterial cell</td>
<td>Bacterial cell</td>
<td>6.5-6.8</td>
<td>Medium</td>
</tr>
<tr>
<td><em>Bacillus mycoides</em> spores</td>
<td>Glass</td>
<td>7.4 ± 3.7</td>
<td>Medium</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> spores 33% RH</td>
<td>Mica</td>
<td>30-40</td>
<td>High</td>
</tr>
<tr>
<td><em>Caulobacter crescentus</em></td>
<td>Glass</td>
<td>590</td>
<td>V. high</td>
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Development of optical based detectors for particle in air analysis

By the early 1990s it became clear that there was an urgent need for biological detectors capable of continuous operation (24 × 7), requiring no liquid reagents and had few false alarms. The Fluorescence Aerodynamic Particle Sizer (FLAPS) was developed by the Defence Research and Development Canada Suffield in collaboration with TSI Inc. (Hairston et al. 1997). The detection principle was based on the theory that live bacterial spore particles fluoresce when excited by UV light, demonstrated later using flow cytometry methods (Laflamme et al. 2005).

Using the FLAPS instrument for studying background air, it was noted that fluorescent particles were not in great abundance, at least at some locations like semi desert environments. To correctly implement statistical techniques in alarm algorithms, there was a need to increase the fluorescent counts obtained over a short time span (in seconds). The best way to accomplish this was to use a virtual impactor (Ho et al. 1999).

As a way to enrich the population of background fluorescent particles for better statistical counting, a prototype aerosol concentrator constructed by Carl Peterson (model XMX, SCP Engineering, St. Paul, MN) was used as a front end to the FLAPS intake. This was a modified version of the original XM2 used for concentrating biological aerosol, as reported previously (Brenner et al. 1988). Ho et al. (1999) employed a smaller version, designed for optimal size, weight and power consumption. It operated at 400-600 l/min, concentrating to 1 l/min delivered to the FLAPS optical intake. Improved particle throughput provided by this setup facilitated a rapid sampling time of 3 seconds. In practice, due to the slow computers available at the time (mid 1990’s), an additional 1 second penalty was incurred for computational and data handling overhead. By this protocol, aerosol data expressed as aerodynamic size distribution and fluorescence intensity could be collected continuously every 4 seconds over long periods unattended by the operator. Fig. 6 shows a modern implementation of a DC motor driven VI with coupling to an optical system.

Ho et al. (2004) characterized ambient aerosol in a forested environment in Umea, Sweden using a FLAPS coupled to a virtual impactor as shown in Fig. 6. With a reference collector system, live bacterial aerosol particles were measured by impaction on growth medium with a slit sampler (Ho et al. 2005). During the week of sampling, there were three major episodes of thunderstorm accompanied by rain. Coincidentally, during the same periods, a significant number of live particles were seen to increase above background levels. At about the same time as the appearance of live particles, the FLAPS also measured concomitant increase in fluorescent particles. Interestingly, these same fluorescent particles exhibited a shift in median size distribution during the same time (Fig. 7). The phenomenon of median size shift to-

![Fig. 6 Prototype FLAPS3 showing a virtual impactor on the left and the particle detection electronics on the right. The coupling of the two systems was accomplished via a spring loaded aerosol transfer tube shown as a golden extension on the right unit. Combining the two units with four clasps resulted in a single one person portable instrument that was designed to be weather proof. In practice, it performed well during a tropical storm. Consequently, it was possible to sample biological aerosol content during rainy periods. This capability opened up a hitherto unprecedented opportunity to explore the presence of biological aerosol content associated with rain episodes.](image-url)
wards the 5-6 µm during thunderstorm related activity has been observed previously by the same author in other locations like Northern Australia and Singapore (unpublished). Recently, Huffman et al (2010) who measured background biological aerosol in Europe using a naturally aspirated FLAPS instrument noted that the appearance of 3 µm particles could be associated with biological content.

In the late 1990s the US Army contracted a newer version of the XM2 to be built, calling it the SCP 1021 (SCP Dynamics, Inc., Minneapolis, MN). It was characterized by solid and liquid aerosols in a chamber using polycarbonate membrane filters as reference sampling method (Kesavan and Doherty, 2001). Working at a flow rate of 1350 l/min, the SCP 1021 registered 25-30% efficiency for particles between 4-5 µm. In contrast, Bergman et al. (2005) performed similar characterization of the (SCP 1026) but used an AGI-30 as reference. For a variety of test aerosol materials they consistently measured close to 80-90% efficiency for particles >2 µm. The discrepancy may be explained by the difference in the reference sampling method.

Complex VI systems

A VI device consisting of complex multi slit inlet was described by Mainelis et al. (2005) and was employed as the aerosol concentrator for a stand alone biological detector. Operating at 1760-3300 l/min, these workers claimed a concentration ratio of $7.5 \times 10^5$ when tested with 3 µm standard latex calibration beads. In contrast, Han and Mainelis (2010) achieved concentration a ratio of $1 \times 10^6$ when employing an
electrostatic precipitator method. From these observations, it would appear VI technology has evolved to become fairly competitive with other technologies.

Sampling viral aerosol

Apart from the work of Brenner et al. (1988) who used the original XM2 to measure viral aerosol particles in the environment, there has been a recent report on the use of VI technology to concentrate and sample virus aerosol (Cooper, 2010). A virtual impactor (XMX/2L-MIL, Dycor Technologies Ltd, Edmonton, Alberta, Canada) operating at a flow rate of 600 l/min was challenged in a 12 m³ chamber with MS2 viral aerosol (Fig. 8). It was reported that sampling efficiency was about 25% when compared to glass impingers at low to medium aerosol concentrations. However, at high challenge concentrations, the VI performance was very close to the reference samplers. In a live avian flu virus sampling campaign, Schofield et al. (2005) using a similar device, captured culturable viruses from an infected chicken barn. This may be a good illustration of the gentle processing characteristics of the VI sampler as viruses have been known to be fragile (Verreault et al. 2008).

Miniaturization of the VI technology

The future in the application of VI technology may be in miniaturization of the components. The Hwang group at Yonsei University in Korea designed and built a micro electro mechanical system (MEMS) based VI with a cut off size at 1 µm (Park et al. 2009). Using Staphylococcus epidermidis as the biological simulant, they reported an impressive collection efficiency of 74-76% based on culturable particles. As discussed earlier, bacteria adhere to surfaces due to inherent stickiness related to polysaccharides. Hwang’s group noted that this was a problem in their narrow liquid channels. Their solution to solving bacterial wall losses was to introduce a 1 kV 1 kHz ac current to the liquid channels. An observed improvement of 12% was obtained (Kim et al. 2010). In another paper, while sampling indoor biological aerosol content, they measured the presence of bacteria, fungi, and actinomycetes (Yoon et al. 2010). It was also demonstrated that culturable cell concentrations were linearly correlated with ATP content.

Conclusions

It can be seen that a variety of machines have successfully integrated VI technologies to liquid chemistries like antibody based reactions or PCR methods. However, this review has revealed major weaknesses in the way detectors are being tested and the use of non standard reference samplers has been mentioned. First, the challenge aerosol used to test the instruments is always presented as a continuous stream. To properly mimic a threat aerosol, the cloud should be presented as short duration puffs. Ho et al. (2010) introduced a method to generate precise puffs of biological challenge aerosol designed to resemble brief emissions encountered in clean room environments. Adopting this method will provide a more meaningful way to determine if a VI equipped device is more effective than the naturally aspirated version. The naturally aspirated mode will serve as the experimental control. Secondly, the instrument must be tested in outdoor environments where background contaminants can properly stress alarming algorithms. There is at present no standard method to test detectors to determine the effectiveness of...
alarming technologies. As mentioned earlier, micro-
particles are naturally sticky, so thirdly, the size
of the aerosol particles should resemble threat mate-
rial consisting of aggregates of individuals as report-
ed by Duncan and Ho (2008). It is generally accepted
that challenge particles should be within the range of
2-5 µm in diameter although some workers may even
go beyond 10 µm (Druett and May, 1952; Thomas et
al., 2008). But large particles are difficult to generate
with any consistency so aiming for the 2-5 µm range is
recommended.

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Author’s short biography

Jim Ho

Jim Ho is defence scientist with the Defence Research and Development Canada at Suffield. He received BSc. and MSc. degrees from McGill University in microbiology and a PhD. from the University of Kentucky in microbial biochemistry. He has been working on the development of biological detection systems since the early 1980’s. In the beginning, he demonstrated the possibility of using LIDAR systems for biological detection. Then he invented an aerosol point biological detector that could reveal if a particle had “live” characteristics. This instrument is currently commercialized by TSI Inc. as models 3313, 3314 and 3317. His current research is focused on characterizing naturally occurring live biological aerosols in different locations around the world. The information gathered has become useful in a variety of areas especially in developing alarm algorithms. He has discovered that minimizing false alarms for detection system is the next most critical phase in biological detection.