

Chapter 2

History of the Early Biodetection Development

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Introduction

The user of a biological detector simply wishes to be notified whether the invisible population of aerosol particles that is about to be inhaled is potentially infectious. On top of that, the military has made it known that there is a requirement for a biological detector that is small enough for handheld applications. The detector has to run on battery power, responds within seconds or minutes to warn of the passage of a potential threat aerosol cloud without false alarms. The information it provides has to be suitable for a medical officer to treat exposed personnel. In addition, the cost and maintenance of each instrument must fall within strict guidelines. At the time of preparation of this chapter (mid-2013), such an instrument did not exist. This review will explore what historically useful contributions have been attempted to solve the problem.

Definition of Terms

There is a need to clarify the term “detection” which is used in the context of biological aerosol threats. The user’s simplistic desire is for an instrument that unambiguously notifies him or her of the presence of an infectious or virulent agent. Knowing this will permit him or her to take evasive/protective action and/or seek for medical assistance. For a variety of technical reasons (testing with animals being one) that will become apparent, it is not feasible to measure infectivity given the technical and time limitations [1]. If time is not an issue, clinical methods can be employed to obtain taxonomic results that only imply if an unknown agent was infectious [2]. The accepted “gold standard” to determine virulence and infectivity is the use of the animal model [3]. There are no detection “short cuts” in the tightly regulated medical and public health environments [4]. Nevertheless, as a compromise, the closest

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to achieving this ideal has been to adopt rapidly responding optical techniques to reveal if the threat agent has “live” characteristics since the ability of an agent to replicate is a prerequisite to infectivity. Following this train of thought, it is clear that current expedient approaches to biological detection cannot be considered as substitute for classical “identification” or “taxonomic” methodologies. This may appear obvious to trained microbiologists but throughout the literature, it is not unusual to encounter papers describing the use of optical approaches that claim to provide capabilities either in identification [5–7] or taxonomy [8]. We respectfully suggest that future workers in biological detection refrain from using the term “identification” if they have not followed the classical medical/clinical methodologies. As an alternative, we suggest using terms such as “segregation” or “sorting” as in segregating or sorting biological particles from background noise material [9].

In the military context, it has been proposed [10] that the primary aim in biological detection is to (1) provide warning to initiate protection and (2) assist the medical officer in prophylaxis and therapy. The latter part of this aim encompasses terminologies in common with the medical profession. Thus, the terms “identification” and “taxonomy” in the clinical setting have very specific meanings as the performance of each task and the results derived thereof can have life and death implications. Simply put, a registered clinical microbiologist has to follow very strict procedures when performing these. At the risk of appearing pedantic, here is the outline of the preliminary identification steps to be executed presuming the availability of a pure bacterial unknown isolate:

1. Determine if the bacterium being identified is Gram-positive or Gram-negative.
2. Is the bacterium round (a coccus) or rod-shaped?
3. Is the bacterium “acid-fast” or not?
4. Does the bacterium produce spores?
5. Does the bacterium grow in the presence of air?

If the answers to these questions were: (1) Gram-positive, (2) rod, (3) no, (4) yes, (5) yes then the bacterium being identified belongs to the genus *Bacillus*. Further biochemical tests will be required to determine the taxonomic species. Even after all these procedures had been performed, there will be uncertainties with respect to the accepted identity and taxonomy of the unknown as has been well illustrated for the pathogen *Burkholderia spp* [11] and a non-pathogen [12]. Using nucleic acid techniques, Danin-Poleg and colleagues [13] demonstrated the difficulties in differentiating between harmless and virulent strains of *Escherichia coli*, *Listeria monocytogenes* and *Vibrio cholerae*. In addition, it should not be surprising that all microorganisms being living things, can evolve with varying rates dependent on environmental pressures [14], making the tasks of “identification” and “taxonomy” much more demanding. In light of these caveats, the strategic approach to biological detection has been proposed in a review chapter [10] and will not be reiterated here. In brief, a biological detector, primarily relying on hardware and software is unlikely to credibly perform “identification” and “taxonomy” tasks to the satisfaction of the medical community.

As in most technological advances, the history of biological detection has been punctuated by a variety of discrete events. At times, these events may be the availability of components, reagents, methodologies or sometimes rediscovery of any of the above. There may be some elements of serendipity associated with the developments where new discoveries are put to use to help solve the biological detection problems. In this review, an attempt will be made to link the events that ultimately led to the results of efforts expended in the quest of detecting the illusive infectious aerosol agents.

Early Attempts Using Light Scatter Optical Particle Counting

In the mid-1800s, John Henry Bell, known as a “British anthrax investigator”, was taking notes of anthrax disease contracted by Bradford wool sorters in the UK. He wrote: “The workers pointed to the fleeces they sorted as the source of the disease and tried to avoid working with “bad” bales of fleece. They suspected that imported fleeces, the alpaca and “van” mohair from the Middle Eastern Levant region, had some kind of dusty poison in them” [15]. Bell himself [16] observed that anthrax infection was most probable from wool with dry dusty content compared to sticky material as shown in this quote: “Alpaca and mohair are not the only infective materials. Many fatal cases have occurred from the manipulation of other dry and dusty hairs and wools. “Greasy” wools are less dangerous; it is probable that the “yolk” in them fixes the infective particles. All hairs and wools (excepting Algerian) may at times contain uncleansed fleeces from animals which have died from anthrax and may occasionally communicate the disease to those who come in contact with them. I have seen such cases from the sorting of British wools, and have heard from my medical friends of others, but the proof is not yet complete”. The reference to “dust” invoked a condition conducive to aerosol generation. The word “aerosol” was coined on page 600, line seven in a paper by Whytlaw-Gray et al. [17] who first introduced the word in print and attributed its origin to Professor F.G. Donnan [18]. So it is not surprising that subsequent workers in biological detection naturally directed their attention and efforts to measuring particles in air, aerosol as is now the common term.

Gentry in a review [19], attributed John Tyndall (1820–1893) to the development of dark field illumination (Tyndall effect) and hence to demonstrating the presence of microorganisms in air. It was noted that the Tyndall effect was also used to detect airborne pollutants. Knowledge from having done these measurements inspired Tyndall to develop a respirator with a charcoal layer to filter out gas vapors and a cotton wool layer to remove particles. His measurements also led to the disproving “Spontaneous Generation” which paved the way to the concept of an explanation for the origin of microorganisms. Tolman and Vliet built an optical instrument called a “Tyndallmeter” primarily to examine smoke particles [20]. It was also shown that light scatter intensity was related to particle size [21].

In an introduction to the biological detector paper by Gucker et al. [22], it was noted that Arthur Guyton [23] at Camp (present day Fort) Detrick between June and October 1945, developed a particle counter. The device caused a stream of particles to impact on a metal plate and apparently particle size was gauged from charge characteristics. It was not sensitive to particles less than about 2.5 μm in diameter. Due to this and other deficiencies, instrument development was abandoned.

In collaboration with the US Army, Gucker's team working in the Chemistry Department of Northwestern University (Evanston, IL), built the first optical particle counter (OPC) for the sole purpose of detecting bacterial spores [22]. It has been known for a long time that anthrax in spore form was a significant military, civilian and agricultural threat [24, 25]. The instrument employed what is now commonly known as sheath flow to control particle transport. The air stream containing the particulate material was confined to the center of a larger sheath stream that passed through the focal point of a dark-field microscope. Particles passing through the system scattered light into a collection lens, eventually producing electrical signals from a RCA 931A photomultiplier tube (PMT). The instrument could just barely resolve the optical signal from a moving bacterial spore in the size range of 0.6 μm in diameter. Of historical significance, the use of *Bacillus globigii* (BG) aerosol as a bacterial spore simulant was first mentioned by Gucker et al. [22]. Being an electronics engineer, he had to rely on scientists at Harvard University to develop the BG aerosol dissemination procedure [26]. For aerosol generation, a medical nebulizer was used. The original glass device, subsequently characterized at the US Army Medical Research Institute of Infectious Diseases, Frederick, MD, was found to produce droplets of about 1.7 μm mass median diameter, whereas a latter day modern plastic model yielded larger ones at about 2.3 μm [27]. It should be noted that the 1947 Gucker instrument had no sizing capabilities; a calibration method for the OPC had not been invented yet.

The limited success of the Gucker instrument in detecting spores relied on the fortuitous availability of certain key components. Shapiro commented that the light source with sufficient brightness to make the Gucker project possible came from a Ford headlight [28]. The 931A PMT came from the Lancaster, Pennsylvania facility opened by the US Navy in 1942 and operated by RCA for the manufacture of radio and microwave tubes. However, Smyth examined a number of 931A PMTs made in the late 1940s and found that their sensitivities were highly variable [29]. Edels and Gambling found that these early production tubes suffered from spatial variations causing signal instability from unfixed sources like inadequately focused aerosol particles [30]. For all these difficulties, it was truly remarkable that Gucker's team could manage to obtain data from their machine. By incorporating 90° light scatter measurement, Gucker and Okonski made improvements to the instrument with the hope of resolving BG and sulfur particles [31]. It was noted that uneven particle dryness caused variability in the signal consistency. The interesting item from this work was that by using a polarizing filter for the light source, BG counts were enhanced.

The next advancement in OPC was registered by Okonski and Doyle [32] which started to measure aerosolized uniform spherical particles of polystyrene and polyvinyltoluene (latex beads) in the size range from 0.1 to 1 μm which had just been produced by Dow Chemical Co. (Midland, MI). It was reported that the particles, when dispersed individually as an aerosol, were ideal for calibration and evaluation of counting instruments because of their high degree of uniformity and low vapor pressures.

By the early 1950s, Gucker moved from Northwestern University to Indiana University at Bloomington where he calculated the Mie scattering properties of spherical particles over a wide range of angles and refractive index [33]. A few years later, he reported on angular scattering characteristics of particles measured with a new instrument. Gucker and Egan measured large portions of the scattering diagram of single aerosol particles (Dioctyl phthalate, DOP) suspended electrostatically in a measuring chamber and viewed by a photocell that moved around the particle (40° to 140°) in 15 or 1.5 min [34]. In later implementation, angular scatter measurement was made more rapidly by using a moving mirror [35]. It is interesting to note that the Harvard University group did not adopt the Gucker detector for their subsequent biological characterization work [36]. However, Gucker's angular scattering measurement instrument inspired Phillip Wyatt who, years later, applied this technique in characterizing bacterial cells in liquid [5].

Wyatt was a physicist who competently exploited historical theoretical particle scattering work of Mie [37], Lorenz [38] and Rayleigh [39] in characterizing batches of bacterial cells in liquid [40]. Many years later, Bronk's group also carried out similar measurements with bacterial suspensions [41]. Bottiger who worked in the same basement laboratory at Edgewood as Bronk, commissioned Wyatt to build a multi-sensor globe for capturing multiple angles of light scatter from a moving stream of aerosol particles [42]. It was discovered that a series of rod shaped bacterial cells produced inconsistent signals when measured at six angles. The authors noted that although 58 channels of light scatter could be potentially monitored, the lack of data handling electronics to match the requirement caused the project to fall short of expectations. The instrument was used to characterize non-biological submicron sized aerosol particles by Peter McMurry's group from the Department of Mechanical Engineering, University of Minnesota in the early 1990s [43]. They concluded that for the small particles examined, the counting efficiency was about 50%. In some instances, it was suspected that aspheric particles compromised data gathering and electronic noise hindered reflective index estimates with the less than optimal seven channels available. Given these experiences, it can be concluded that the power of multiple angle light scatter as a way for bacterial aerosol characterization had not been fairly tested. The biggest challenge that future workers in this field will encounter is that biological particles are inherently aspheric as discussed in the next section. Thus, attempting to fit light scatter data from randomly shaped material can become difficult to manage.

Bacterial Shape Analysis

Microbiologists have known for a long time that bacteria display a variety of cell shapes, including round, rod, spiral and amorphous. In a recent review, Young described over 24 morphological structures that bacteria may assume [44]. Maintenance of cell shape is vital for cell growth and cell division in most bacteria. The distinct shape of most bacteria is retained by a peptidoglycan layer, enveloping bacterial cells as a single structural macromolecule [45]. Analogous to actins in eukaryotes, the bacterial tubulin, FtsZ (standard gene designation has no full name equivalent), has an important function in cell division [46] and contributes to cell morphogenesis. FtsZ protein forms a ring-like structure at mid-cell, which serves as a scaffold for other cell division proteins. Another bacterial cytoskeletal protein MreB determines the length of the short axis of the cell, whereas Mbl determines the length of the long axis of the cell. Both MreB and Mbl form a helix or filament just beneath the cytoplasmic membrane along the long axis of the cell in *B. subtilis* [47]. It can be safely assumed that the bacterial shape is fundamental to the distinctiveness of the native or non-mutated version of the species. It is natural for those workers with microbial training to exploit this property in biological detection.

In the mid-1980s Cox who later wrote a book on biological aerosols [48] asked Bexon who worked on aerosol holography [49] how to design an instrument for measuring the shape of a single bacterial aerosol particle. Bexon, a physicist, probably had Mie in mind, he theorized that by monitoring three axes of light scatter, particle shape resolution may be possible. Cox commissioned the Laser Systems Research Group at the Hatfield Polytech, Hatfield, Herts, UK to build a small battery powered “shape analyser” (SA). Eventually, a much larger version than the one initially envisioned was built. In characterizing spheres and fibers the term “asymmetry factor” was introduced [50]. The instrument was demonstrated to produce spherical scatter data close to Mie predictions [51]. However, it was discovered that the SA instrument was mostly suited to characterizing elongated fibers rather than bacterial shapes [52–54]. In the late 1990s the Hatfield group combined a 266 nm excitation light source to the original SA concept with the hope of supplementing shape analysis with intrinsic protein fluorescence to provide more robust biological particle resolution [55]. However, by mid-2000s, the group abandoned the SA concept altogether to concentrate their efforts on using 260–280 nm excitation to produce fluorescence signals for characterizing proteins. Much later, 370 nm excitation, pioneered by Suffield in the 1990s, was adopted for probing biological metabolic molecules [56]. More recently, the SA concept was used to characterize atmospheric ice crystals [57]. As a footnote, in a recent paper describing multiple angle light scatter by bacterial spores, it was concluded that the measured signals did not correspond to modeling predictions using T-matrix to simulate a spore with three different particle shapes [58]. Gogoi et al. reported that for complex particles like fresh water diatoms, multiple angle light scatter data did not correlate to Mie predictions [59]. Interestingly, in 1978, Latimer setting out to define “asphericity” of single particles, concluded that data reliability could be compromised by random

particle orientation and irregularity of shapes [60]. After over a quarter century of putting Mie to the test, the consensus is that relying on light scatter measurement of single biological particles as a distinguishing characteristic for detection purposes will remain illusive. Nevertheless, the technology has been actively used to detect sudden changes in the aerosol composition and to trigger subsequent sampling and analysis steps. An artificially made aerosol will introduce a fraction of aerosols in the natural background that can be detected by the SA technology. Classification algorithms can continuously analyze the scatter profile and detect deviation from normal conditions.

Origins of Luminol

Luminol (5-amino-2, 3-dihydro-1-4-phthalazinedione) was first synthesized in Germany in 1902, but the compound was not named luminol until the late 1920s. Metcalfe and Quickenden [61] attributed much of the early studies to H.O. Albrecht [62] who synthesized and worked with the compound in 1928 but it was difficult to obtain the original paper for verification. Subsequent workers found that the compound produced a strong glow (chemiluminescence) in the presence of an iron contain material like hemoglobin [63]. Neufeld et al. from the US Army Biological Laboratories, Fort Detrick, Frederick, MD, discovered that the compound was effective for measuring other iron bound proteins [64]. Soon after the appearance of this paper, the Russians also published the paper on this subject [65]. Later, Miller and Vogelhut reported that the assay had a limit of sensitivity of about 10^5 to 10^6 viable bacteria per ml [66]. At about this time, in the early to mid-1970s, probably based on these and other studies [67], the US Army contracted Bendix, Environmental and Process Instrument Division in Baltimore, MD to manufacture a biological detector (XM19) based on the luminol chemistry (Fig. 2.1).

As a historical footnote, there was a similar Russian instrument that was probably produced slightly ahead of the XM19 although verification information is difficult to obtain.

The XM19 was made possible by a number of converging technologies. To detect the presence of hazardous biological particles in air, it was necessary to concentrate the material sufficiently for downstream measurements. Marple from the University of Minnesota reviewed the historical fundamentals of virtual impactor (VI) technologies that were used for such a purpose [68]. Carl Peterson (SCP Dynamics, Inc., Minneapolis, MN) manufactured the VI used for the XM19 that had a flow rate of 1000–1350 l/min at the intake end and 1 l/min at the output or delivery end. A companion sample collector called XM2 (Fig. 2.2) used for aerosol sampling, was described in the open literature [69]. A modern version called the “SCP 1021” was characterized by Kesavan and Doherty [70] and more recently by Bergman et al. [71].

In the XM19, the delivered aerosol particles were impacted on a moving plastic tape, part of a cassette transport system [72] that was calibrated to shift from

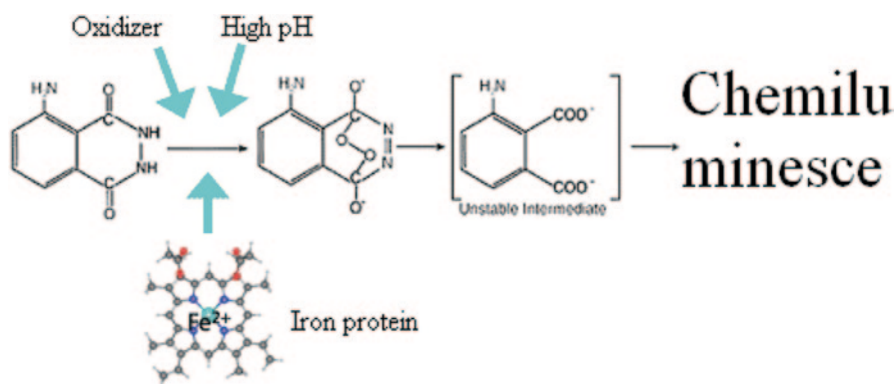


Fig. 2.1 XM19 biological detector showing virtual impactor, tape cassette, liquid chemistry, electronic sensor and analogue electronic panel

the collection site to the liquid chemistry site, timed precisely to ensure sufficient particles were trapped on the tape surface. At the liquid chemistry site, luminol reagents were applied to the particles as a flowing stream. The swirling liquid stream dislodged some or most of these particles off the tape surface. Clever plumbing was employed to extract a fraction of the biological material; delivery rate was designed to ensure an appropriate reaction time, eventually presenting the reacted material to

Fig. 2.2 XM2 was a liquid based sampler that sequentially impinged aerosol particulates into two liquid containing plastic containers. The system could take a series of two samples after being triggered by the XM19



a PMT for chemiluminescence detection. The optical signal strength could be translated to indicate if there were sufficient particles measured to warrant an alarm. On alarming, a trigger was transmitted to a companion VI-based liquid impingement collector (XM2) as shown in Fig. 2.2. Under chamber test conditions, the instrument couple performed as expected when presented with BG aerosol. However, when tested outdoors, random false alarms became an issue [73]. In hindsight, it became clear why the machine responded to the non-specific background aerosol materials. Subsequent to the inception of the instrument design, it has been known that air contains a variety of iron containing particles from marine and terrestrial sources [74]. Most particles in ambient air may have a soil origin and it has been shown that soil humic acid forms a complex with iron providing substrate for the luminol reaction [75]. After a few years of environmental testing in a variety of locations, the US Army rejected the XM19 for the task of biological detection [76]. Currently, luminol has found acceptance in forensic applications for detection of blood splatter in crime scenes where the occasional false positives can be mitigated by other technologies [77]. By present day standards, the reagents are relatively inexpensive and in forensic applications, its high sensitivity compensates for its other deficiencies.

Fig. 2.3 XM19 (*left*), the detector and XM2 (*right*) the sample collector were designed to work electrically linked together. When the detector triggered an alarm from analysis of a biological aerosol presence, a signal was sent to the XM2 to initiate the collection of liquid samples. Note the provision of handles for mobility. In practice, hand carrying each unit was a two man effort



Lessons Learnt

Some valuable lessons could be gleaned from this financially significant failure. First, measuring one parameter, biological iron, as the sole determinant for the presence of a biological threat was insufficient for minimizing false alarms, in view of the ubiquity of the interfering material in ambient air. Second, the XM19 relied on continuous operation to be effective, thus required 24/7 consumption of liquid reagents that over time, may become expensive and have limited shelf live. Support and maintenance of a network of systems can translate into enormous logistical and cost requirements. Third, the combined weight of the XM19 and XM2 exceeded 150 kg, probably more than an average man can comfortably transport (Fig. 2.3). As the two VI concentrators operated a high flow rates, power requirements became significant. Fourth, for developing more sophisticated alarming technologies, there was an urgent need to understand background air, for example, detailed knowledge of aerosol particle characteristics.

Revisiting Light Scatter Measurements and Optical Particle Counting

For studying ambient aerosol characteristics, the aerodynamic particle sizer (APS, TSI St Paul, MN) that was commercialized in 1982 [78] with real time software driven data display, turned out to be a good choice. In a review, Baron has provided some background on the development of the APS in addition to describing the instrument characteristics with respect to solid and liquid particles [79]. Volckens and Peters exploring the measurement efficiencies reported that for solid particles, counting efficiencies ranged between 85% and 99% [80]. Ananth and Wilson calculated correction factor as a function of particle density and APS derived

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