A REVIEW OF TECHNIQUES FOR THE DETECTION OF BIOLOGICAL WARFARE AGENTS

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ABSTRACT

Biohazards represent an important issue in the field of security, both for the destructive potential and the psychological, economic and social impact that the use of biological agents for biowarfare could have on populations. Early identification of an intentional biological event is essential to ensure correct management and response to the emergency. Much effort for the development of innovative equipment that permit prompt and remote detection of biological warfare agents are needed to achieve this goal. In this work, the different detection systems suitable in the CBRN context for biological agents will be analyzed, focusing on non-specific and specific point-detection systems, and stand-off detection systems, evaluating the pros and cons of each technology.

Keywords: *Biological warfare agents; non-specific and specific point-detection systems; stand-off detection systems; sensitivity and specificity; quickness of response.*

1.   INTRODUCTION

In the last few decade, concerns on intentional use of biological agents (bacteria, viruses, fungi, toxins) as weapons have increased along with the terrorism global alert. Among the non-conventional threats, biohazards is considered as an extremely demanding challenge for chemical, biological, radiological and nuclear (CBRN) experts, due to difficulties concerning detection and identification of biological agents (Cenciarelli *et al.*, 2013a), and lack of suitable prophylactic and therapeutic measures for many of these pathogens. Biohazards are caused from the dispersion in the environment of a microorganism or toxin, with the consequent possibility to cause a communicable disease in affected people (except for toxins, which are not infectious). Such an event could occur through natural spread of an infectious disease (i.e., influenza epidemics or the ongoing Ebola virus disease outbreak in West Africa), accidental dispersion of an agent (i.e., an accident in a laboratory where biological agents are usually used), or intentional dissemination as a terrorist act (Cenciarelli *et al.*, 2013b, 2014a).

Biological warfare refers to the deliberate use of microorganisms and toxins, generally of microbial, plant or animal origin, to produce diseases in humans, animals and plants (DaSilva, 1999). The intentional release of aggressive biological agents aims to strike a large number of people, causing serious illnesses and increasing their spread. Biological weapons (except toxins) have, in fact, an intrinsic characteristic; they are able to multiply in a host organism and be transmitted to others, thereby causing unpredictable effects in the population, both in terms of victims and geographical spread (Rotz *et al*., 2002). Easy dissemination and high lethality of some biological agents (CDC, 2015) make it difficult for prompt detection and identification of a biological attack (Cenciarelli *et al.*, 2013a). Biological agents can remain undetected for hours, days or even weeks before the onset of the illness. Without any immediate sign of dissemination (as occurred in the event of anthrax letters) and before disease confirmation by health authorities through clinical tests, a biological event can only be identified by monitoring systems. Great importance should be addressed to this aspect because while tools for immediate detection of chemical and radiological agents are largely available (Jopling, 2005; Sferopoulos, 2009), preparedness towards biological agents is still low, with just some rare exceptions (Kaszeta, 2012; Cenciarelli *et al*., 2013a). Traditional methods for detection and identification of biological agents lack the speed and sensitivity to be applied in the field because they cannot provide results in real time (Iqbal *et al*., 2000).

Detection systems must be highly sensitive, being able to detect extremely limited amounts of biological particles. For example, a concentration of 100 particles/L of *Bacillus anthracis* or only 10 particles/L of *Francisella tularensis* can infect a person (Primmerman, 2000). Moreover, such a system should be able to discriminate pathogens from other harmless biological and non-biological components that are part of the environmental background (e.g. diesel particulates, pollen, dust), to achieve a low false-positive rate. Normally, ambient particle concentration exceeds the predetermined detection concentration of biological agents (Greenwood *et al*., 2009). A further quality that must be evaluated is the quickness of response, because prompt detection is the key for an efficient intervention (Primmerman, 2000). These requirements make it very easy to understand how hard it is to develop detection systems that allow for effective detection of biological agents.

It must be considered that some promising tools for detection of biological agents have been developed and tested, especially by the army. However, they are complex systems, the use of which requires special formations for their proper execution and maintenance. Moreover, they tend to be very expensive. In recent years, many companies have focused their attention on the development of biodetection tools that are less expensive and easier to use than the products of military source (Ozanich *et al*., 2014).

Detection systems can be divided into two broad categories; point-detection and stand-off detection systems. Point-detection systems are able to sense biological particles in a short range (from centimeters to some meters), while stand-off detection systems have the capability to detect biological particles from far away, even for some kilometers (Švábenská, 2012).

In this paper, several technologies for the biological agents detections in the CBRN context were investigated, evaluating both non-specific and specific point-detection systems and remote (stand-off) detection systems; for each system advantages and disadvantages were assessed.

2.   POINT-DETECTION SYSTEMS

Point-detection systems may be specific or non-specific, depending on their ability to discriminate a definite biological agent once the analysis is performed. Non-specific detection systems are only able to determine if a biological agent is present, without providing any identification. On the other hand, specific point-detection systems are able to return an identification of the biological agent.
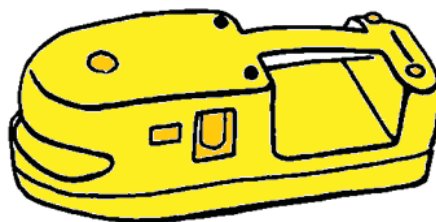
2.1   Non-Specific Point-Detection Systems

- **Particle sizers'** operating principle is based on counts of the relative number of particles included in a predetermined size range (typically 0.5-30 μm) (Pazienza *et al.*, 2014). One of the most diffused particle sizers is High Volume Aerodynamic Particle Sizer (HVAPS), in which particles are exposed to a constant flow of concentrated air. Within the aerosol, particles will accelerate with different rates, depending on their size. Thus, the acceleration will be higher for smaller particles. This technology, due to a laser measuring device, provides information about the number, size and distribution of particles. However, such device does not permit distinction among biological and non-biological aerosols.

- **Fluorescence based systems** exploit the properties of endogenous fluorophores to detect biological agents through bioluminescence (Carestia *et al.*, 2014). This method consists of the excitation of molecular components widely present in biological materials (such as the aromatic aminoacid tryptophan) with light beams (usually in the ultraviolet (UV) spectrum). With the light emission after excitation, these approaches could be used for non-specific detection of biological agents in unknown samples exploiting emission of a common fluorophore. Among the detectors that use fluorescence measurements, the most prominent is Fluorescent Aerodynamic Particle Sizer (FLAPS) (Figure 1). It is an aerodynamic particle sizer, like the above-mentioned HVAPS, provided with an additional UV laser. A variant of FLAPS is Ultra Violet Aerodynamic Particle Sizer (UVAPS) that uses, for sizing, the particles time of flight, light dispersion and UV fluorescence intensity to detect biological agents in air samples. Unlike FLAPS and UVAPS, Biological Aerosol Warning System (BAWS) technology does not provide a count of particles, but can detect in real time and discriminate particles of biological agents from other airborne particles naturally present as environmental background (Huffmann *et al.*, 2013).

- **Viable particle size samplers (impactors)** operate by accelerating an air flow through a nozzle before deflecting it against an impact surface maintained at a fixed distance. Larger particles that are not able to follow the flow due to their inertia will be separated from smaller particles. Small particles exit the sampler, passing through distinct stages, each one containing progressive dimension holes that allow the diffusion of particles according to their size and the collection on a specific surface. The collection plate is generally represented by a Petri dish containing selective agar to allow the growth of specific microorganisms. After an incubation period, typically 24-48 h, the number of colonies grown on each plate is evaluated (Xu *et al.*, 2013).

Figure 1: Schematic representation of a Fluorescent Aerodynamic Particle Sizer (FLAPS).
(Adapted from http://www.tsi.com)

- **Virtual impactors** represent a class of tools similar to the viable particle size samplers, except for the collection plate, which consists of a probe that the larger particles can penetrate. Moreover, once reaching the final stage, particles will flow into a liquid matrix, with the production of a highly concentrated liquid sample. The BioVIC aerosol collector is a device able to pre-concentrate the flow of air, by suspending a large number of particles in a reduced volume of liquid, in a small flow of air or on a solid surface for subsequent detection by a sensor. It can be associated with polymerase chain reaction (PCR) technology, optical fluorescence-based sensors, mass spectrometry or flow cytometry. An evolution of BioVIC is the BioCapture BT-500 air sampler (Figure 2), which is a portable instrument that collects airborne samples to quantify the particulate concentration. Pathogens are collected and concentrated in a liquid sample for further analysis carried out through the rapid identification of cellular components, nucleic acids or other identifiable components in liquid matrix (Kesavan *et al*., 2011).

Figure 2: Schematic representation of a BioCapture BT-500 air sampler.
(Redrawn and adapted from http://www.htds.fr)

2.2   Specific Point-Detection Systems

- **Molecular biology techniques**, including PCR, is the most common method to amplify small amounts of genetic material), allowing for the detection of biological agents (bacteria, bacteria spores or viruses only; toxins do not possess genome) (Iqbal *et al*., 2000). The main limitation to this technique is the requirement for a prior knowledge of the biological

agent analyzed, due to the need of specific primer sequence for the nucleic acid amplification. In addition, each reaction is generally specific for a single agent, except for multiplex PCR, by which the analysis of several agents at once is possible (Greenwood *et al.*, 2009). Specific probes may be applied as a complement to the PCR. This technique detects the presence of a specific gene sequence in the sample, by exploiting the interaction among complementary sequences. Nowadays, such an approach finds common application in DNA microarrays (Lee *et al.*, 2008; Splettsosser *et al.*, 2010), based on the simultaneous hybridization of thousands of specific gene sequences. Briefly, different DNA sequences are deposited at a distance of a few hundred microns on a chip, usually made of glass, consisting of an array of microscopic DNA probes. The hybridization process is highly selective, specific and sensitive. A large number of target sequences will be available on a single support, potentially providing more complete information than that of a simple PCR, although depending on it for signal intensity (Call *et al.*, 2003). However, providing increased information per unit time is not the only advantage offered by microarrays. A significant reduction in the time of analysis, small sample volume and reagents required are other important elements in favor of this technique (Ivnitski *et al.*, 2003).

- **Flow cytometry** evaluates both the physical and chemical features of an air flow when it runs through a testing point. This instrument counts and measures the size of particles dispersed after liquid phase concentration using a laser diffraction system. Generally, a fluorescent dye that reacts with biological components*,* such as DNA, is added to the sample before the measurements. Flow cytometers are very complex systems provided with sophisticated mechanisms that permit the analysis of thousands of cells in a few seconds. Among these, Mini-Flow Cytometer and Fluorescence Activated Cell Sorting (FACS) Caliber (Becton Dickinson) are the most commonly used.

- **Mass spectrometry** is an analytical technique that provides information about structure and molecular weight of biological agents requiring minimal samples amounts (order of nanograms). Generally, it is applied in combination with separating techniques, such as gas chromatography and High-Performance Liquid Chromatography (HPLC) (Figure 3) and works by ionizing molecules to generate molecule fragments, whose pattern constitutes the mass spectrum, which is a plot of the ion signal as a function of the mass-to-charge ratio. This technique requires samples in gaseous state. Examples of such tools are Matrix-Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) and Chemical Biological Mass Spectrometer (CBMS) (Figure 4) (Wieser *et al.*, 2012; Laskay *et al.*, 2012).

- **Immunoassay technologies** allow for the identification of biological agents using the specific binding of antigens with specific antibodies forming a detectable complex (Peruski & Peruski, 2003). Generally, these assays provide a response in a short time. Their sensitivity can vary depending on the sample medium, suspected agent and specific device (Greenwood *et al.*, 2009). Hand-Held Immunochromatographic Assays (HHAs) are disposable kits that, working on the principle of antigen/antibody interaction, show their results colorimetrically, with the same mechanism of a pregnancy test. They can provide both qualitative and semiquantitative response about a specific agent. These devices are