4. BIOLOGICAL DETECTION SYSTEM COMPONENTS

The effective detection of biological agents in the environment requires a multicomponent analysis system because of the complexity of the environment. Other variables contributing to the effectiveness of detection of biological agents are the detection process itself and the efficient use of consumables in the field. Biological agent detection systems generally consist of four components: the trigger/cue, the collector, the detector, and the identifier. Figure 4–1 shows a flow diagram for a typical point detection automated architecture system. The function of these components is described in the remainder of this section, while section 5 will provide representative examples of each component.

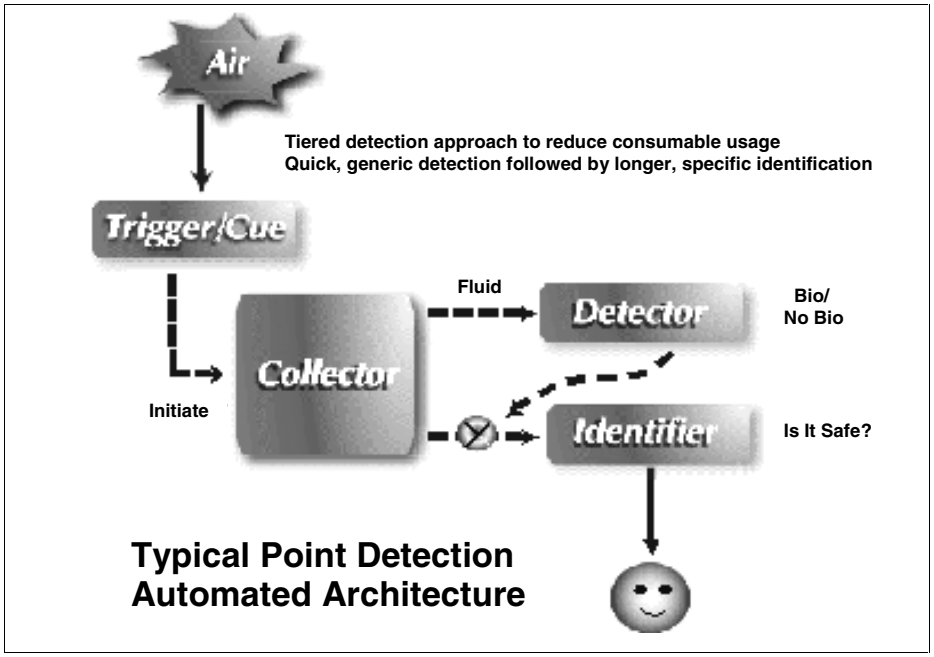

Figure 4–1. Typical point detection automated architecture (with a combined trigger/cue)

4.1 Trigger/Cue

Trigger technology is the first level of detection that determines any change in the particulate background at the sensor, indicating a possible introduction of biological agents. Detection of an increase in the particulate concentration by the trigger causes the remaining components of the detection system to begin operation. The trigger function typically provides a means of continuously monitoring the air without unnecessary use of consumables, thus keeping the logistical burden of biological agent detection low.

To reduce false positives (alarm with no biological agent) and false negatives (no alarm with agent), many detection systems combine trigger technology with a second detector technology (such as fluorescence that provides more selectivity) into a single technology known as cueing. Most effective cueing technologies can detect airborne particulates in near real time and can discriminate between biological agent aerosol particles and other particles in air, avoiding

unnecessary system activation. For example, a cueing device monitors the air for particulates as does any other trigger device. When the particulate concentration increases, the cue determines if the particulates are biological in nature. The cue device generally uses a fluorescence detector to make this determination. If the particulates are found to be biological, the cue device activates the collector for sample collection.

4.2 Collector

As discussed in section 3.4, sampling of the biological agent is a crucial part of the identification system. The effective dose for some agents is extremely small; therefore, highly efficient collection devices must be employed. One type of collector pumps large volumes of air through a chamber where the air mixes with water. The water scrubs all the particulates from the air, resulting in a sample containing particulates suspended in water. Once collected in the water, the sample is further concentrated by evaporation of a portion of the water. After concentration, the sample moves into the analytical section of the biological agent detection system.

4.3 Detector

Once a sample has been collected/concentrated, it must be determined if the particulates are biological or inorganic in origin. To accomplish this, the sample is passed to a generic detection component that analyzes the aerosol particles to determine if they are biological in origin. This component may also classify the suspect aerosol by broad category (e.g., spore, bacterium, toxin/macromolecule, or virus). In its simplest form, the detector acts as a "gateway" for further analysis. If the sample exhibits characteristics of biological particles, it is passed through to the next level of analysis. If the sample does not exhibit such characteristics, it is not passed to the next level of analysis, thereby conserving analytical consumables.

It is important to note that detection has traditionally taken place after the trigger function. For example, an aerosol particle sizer (APS) triggers, then a detector (e.g., flow cytometer) examines the aerosol for biological content. Many of the newer detection technologies combine the trigger and detection functionalities into a single instrument, creating a cueing instrument. As described in section 4.1, the cue first detects a rise in particulates then determines if the particulates are of biological origin. If the sample is biological, the collector gathers a sample and passes it directly to the identifier.

4.4 Identifier

An identifier is a device that specifically identifies the type of biological agent collected by the system. Identifiers are generally limited to a preselected set of agents and cannot identify agents outside of this set without the addition of new identifier chemistry/equipment or preprogramming. Because the identifier performs the final and highest level of agent detection, it is the most critical component of the detection architecture and has the widest variety of technologies and equipment available. The information obtained from the identifier is then used to determine protection requirements and treatment of exposed personnel.

5. OVERVIEW OF BIOLOGICAL AGENT DETECTION SYSTEM TECHNOLOGIES

The applicability of biological agent detection equipment to emergency first responders will depend on the characteristics of the detection equipment, the type of biological agent to be detected, and the objective of the emergency first responder unit. Good analytical results from the various analyzers will depend on the ability to effectively sample the environment and deliver the biological agent to the analyzer.

Biological detection systems are currently in the research and early development stages. There are some commercially available devices that have limited utility (responding only to a small number of agents) and are generally high cost items. Because commercially available biological warfare (BW) detection systems and/or components exhibit limited utility in detecting and identifying BW agents and are also costly, it is strongly recommended that first responders be very careful when considering a purchase of any device that claims to detect BW agents. This is a very different situation when compared to chemical detection equipment; there are various technologies for detection of chemical agents and toxic industrial materials (TIMs) that can be purchased by the emergency first responder. One reason for the lack of available biological detection equipment is that detection of biological agents requires extremely high sensitivity (because of the very low effective dose needed to cause infection and spread the disease) and an unusually high degree of selectivity (because of the large and diverse biological background in the environment).

Another reason for the lack of biological detection equipment is that biological agents, compared to chemical agents, are very complex systems of molecules, which makes them much more difficult to identify. For example, Ionization/Ion Mobility Spectrometry (IMS), an excellent (though expensive) system for collection, detection, and identification of chemical agents, cannot detect or discriminate biological agents in its present form. In fact, the need for high-efficiency collection and concentration of the sample, high sensitivities, and high selectivities make all chemical detectors in their current form unusable for biological agent detection.

Because of the need for high selectivity and sensitivity, the biological detection systems are necessarily complex devices consisting of various subunits. Each subunit performs a specific collection, detection, and identification task. In this section, the various units and subunits that make up biological agent point and standoff detection systems are described. Specifically, section 5.1 discusses the separate technologies utilized with point detection, section 5.2 discusses standoff technologies (both short range and long range), and section 5.3 addresses passive standoff detection.

For reference only, examples of the size and complexity of integrated biological detection systems are presented in figure 5−1 and figure 5−2. They are the Biological Integrated Detection Systems (BIDS) from the United States and a cutaway picture of the Integrated Biological Integrated Detection System from the United Kingdom, respectively.

Figure 5-1. Biological Integrated Detection System (BIDS)

Figure 5-2. Cutaway of the UK Integrated Biological Detection System (IBDS)

5.1 Point Detection Technologies

Point detectors are those sensors that must be in the aerosol plume or have the suspect biological agent introduced into/onto them for sensing. Point detection systems have traditionally encompassed the following components: trigger/cue (nonspecific biological agent detectors), sampler/collector, and identifier (specific identification technologies).

5.1.1 Trigger/Cue (Nonspecific Biological Agent Detectors)

The function of the trigger is to provide early warning that a change in the background air has occurred. Operation of a trigger requires establishing background aerosol levels in a specific location and then sensing that an increase in the aerosol particle count in the background has occurred. A trigger is nonselective and does not identify the organism but only indicates a change in the background aerosol level. Since a trigger is nonselective, a detector is required if there is no cue.

A cueing device is first able to determine when there is an increase in particulates and then is able to distinguish between concentrations of biological aerosols and nonbiological aerosols (nonspecific biological agent detection). Descriptions of several detector technologies are presented in section 5.1.3.

Brief descriptions of trigger/cue technologies are presented in the section below.

5.1.1.1 Particle Measurement

One technique used for nonspecific detection is counting the relative number of particles in specific size ranges (typically 0.5 μm to 30 μm). A variety of technologies are used for particle monitoring and/or counting, but aerodynamic particle sizing has been directly applied to field biological agent detection. Several examples of particle measurement technologies follow.

Aerodynamic Particle Sizing (APS): The particle-laden air stream is drawn into the APS device through a flow nozzle, producing a controlled high-speed aerosol jet. During the measurement

period, the air velocity remains constant but because of the different sizes of the individual particles within the jet, they accelerate at different rates based on their relative sizes (smaller particles accelerate faster than larger particles). A laser beam measures the time of flight of the individual particles.

High Volume Aerodynamic Particle Sizer (HVAPS): The HVAPS passes an accelerated, concentrated air stream past a laser-based particle counter to obtain aerosol particle size distribution and concentration. This instrument cannot discriminate biological from non-biological aerosols.

Met-One: The Met-One is a compact, low-power aerosol particle sizer and counter about the size of a large, hand-held calculator. This device is available commercially and is typically used to monitor clean rooms. The Met-One draws an air sample through a laser-illuminated sample volume where airborne particles scatter light. The light scattered by individual particles is then detected using a photodiode. Like the HVAPS, the Met-One looks for a statistically significant rise in aerosol concentration over background; however, the Met-One is not able to resolve the particle sizes as finely as the HVAPS. The Met-One gains its size and weight savings through a combination of low airflow and use of a low-power, diode laser.

5.1.1.2 Fluorescence Methods

Fluorescence approaches involve excitation of molecular components of a material with light, usually in the ultra violet (UV) region of the spectrum. The excited component spontaneously reverts to an unexcited state followed by emission of light at different wavelengths. Because the emission spectrum is specific to the molecular component being irradiated and the excitation wavelength, this phenomenon can be exploited in detection of biological material (biofluorescence). Biofluorescence-based techniques generate data from only some specific molecular components of biological material, allowing it to be a tool for nonspecific agent detection by providing the emission spectrum of a common material (i.e., tryptophan) when an unknown sample is irradiated.

The two types of fluorescence measurement approaches are primary and secondary. In primary biofluorescence, some common, naturally fluorescent component of biomaterials, such as tryptophan (an amino acid building block of protein), is measured. Secondary fluorescence methods involve introducing (tagging) a special fluorophore (i.e., fluorochrome stain) to the sample before UV irradiation. Secondary methods require a longer measurement time and add complexity to the measurement process. Several devices that use biofluorescence technologies are included in the remainder of this section.

Fluorescent Aerodynamic Particle Sizer (FLAPS): FLAPS is an Aerodynamic Particle Sizer (APS) that has been modified to include an additional laser (blue or UV wavelength) that provides for aerosol particle fluorescence in addition to standard particle size information. Besides obtaining the aerodynamic particle size, the laser's signal acts as a trigger to open a time window in which to look for particle fluorescence. The information obtained from this technology will be more specific than the current standard particle size and number density results.

The FLAPS II device is part of the Canadian Integrated Biological Agent Detection System (CIBADS), a.k.a., the 4WARN detection suite. The CIBADS is an integrated system of components developed by the Canadian Ministry of Defense that currently contains a detector/trigger function, sample collection function, meteorological instrumentation, and communications equipment. A picture of the FLAPS II is presented in figure 5–3, and the 4WARN system from Canada is presented in figure 5–4.

Figure 5-3. FLAPS II (component of the Canadian 4WARN System)

Figure 5-4. Canadian Integrated Biological-Chemical Agent Detection System (CIBADS)/4WARN

A variation of the FLAPS particle sizer is the Ultra Violet Aerodynamic Particle Sizer (UVAPS) that uses time-of-flight particle sizing, light scattering, and UV fluorescence intensity to nonspecifically detect biological agents in air samples. The UVAPS (as well as the FLAPS) is commercially available from TSI Inc., Particle Instruments.

The Biological Aerosol Warning System (BAWS) is effective as a trigger/cue technology. The BAWS uses a micro-laser-based system that analyzes two biological fluorescence wavelengths to determine if an unusual biological event is happening. The BAWS does not count aerosol particles. It can detect in real time and can discriminate biological agent aerosol particles from other particles in the air to avoid false triggers.

A technique called Portable Biofluorosensor (PBS) was used during Operation Desert Storm. The technique used UV light from a xenon flash lamp to excite airborne aerosols and aerosols dissolved in water. The excitation wavelength minimized interference from dust, exhaust, etc., but did not eliminate false positives. Liquid samples containing spores provided better analysis results than airborne samples.

The Single-Particle Fluorescence Counter (SPFC), developed by the Naval Research Laboratory (NRL), employs continuous airflow across a 780 nm laser-diode beam, resulting in light scattering from individual aerosol particles in the air. The total intensity of scattered light is

measured, and particle size is calculated. This event also triggers a 266 nm UV laser pulse that causes fluorescent particles to emit light at a different wavelength (i.e., the particles fluoresce).

5.1.2 Samplers/Collectors

Since an extremely low airborne concentration of biological agents can be difficult to detect but still cause severe effects, a device to concentrate particles/aerosols in the air stream is needed. A collector/concentrator samples the atmosphere and concentrates the airborne particles into a liquid medium for analysis. Several types of samplers/collectors have been evaluated for biological agent detection. The principal differences between collection for biological agent detection and other types of aerosol or particulate sampling are (1) biological agent sampling is normally targeted at living organisms, so the sampling techniques must preserve and not harm the collected sample; (2) most biological detection and identification technologies require a liquid sample, so the collection must be from an aerosol or particulate in a liquid; and (3) the liquid sample must be highly concentrated and available for rapid analysis since response time is critical.

A collector is most useful when it is part of a detection system. When the collector receives a signal from a trigger indicating a change in the background level, an air sample is collected, and airborne particles are concentrated into a liquid medium.

The efficiency of a collector at capturing and concentrating aerosol samples typically affects several downstream functions. In virtually all systems, the collectors feed into the identification component of the biological detection system and also provide the samples that are used for confirmatory identification and forensic analysis.

Collectors can be broadly divided into two groups. One group contains collectors that are large and consume much power. These collectors, on the whole, have a high collection and concentration efficiency and are candidates for detection systems that operate well away from the line or point of agent release. The other group contains those collectors that consume little power, are hand-portable, and have relatively low collection and concentration efficiencies. Whereas these collectors would work well in high agent concentrations (e.g., near the point or line of release, or perhaps indoors), they would fail to provide an adequate sample to downstream instruments. It should also be noted that collectors significantly contribute to the overall weight, size, and power requirements of a detection system.

Examples of sampler/collector technologies include Viable Particle Size Samplers (Impactors), Virtual Impactors, Cyclones, and Bubblers/Impingers.

5.1.2.1 Viable Particle Size Samplers (Impactors)

A conventional impactor operates by accelerating an air stream of particles through a nozzle and diverting the air stream against an impaction plate maintained at a fixed distance from the nozzle. The larger particles are unable to follow the fluid streamlines (air in this case) because of their large inertia; smaller particles follow the fluid streamlines and exit the sampler.

The impactor usually has multiple stages and each stage contains a number of precision-drilled orifices that are a constant size for each stage. Particle laden air enters the instrument, and the airborne particles are directed towards the collection surfaces by the jet orifices. Any particle not collected by a specific stage follows the stream of air around the edge of the collection surface to the next stage. The collection plate is typically a petri dish with selective agar (selective to a specific organism). The plates are incubated (typically 24 h to 48 h) and after incubation, the number of colonies on each plate are counted.

5.1.2.2 Virtual Impactors

A virtual impactor is similar to a conventional impactor but uses a different impaction surface. The flat plate of the conventional impactor is replaced by a collection probe, and the larger particles penetrate the collection probe instead of striking a flat plate. By properly controlling the airflow in the impactor, it is possible to collect particles in a specific size range. In addition, the final stage can then aim the particle stream onto a liquid, resulting in a highly concentrated liquid sample.

The Liquid Sampler (PEM-0020) with carousel is manufactured by Power Engineering and Manufacturing, Inc. The device uses virtual impaction to collect and concentrate airborne particles onto liquid film. The operator can select the number of samples to be collected (up to 10) and can choose from several preprogrammed sampling protocols that vary the volume and the collection time for each tube. Initiation of the sample collection is by external trigger or manual push button. The unit automatically repositions the carousel at the end of the collection cycle. The entire carousel can be quickly removed and replaced.

The BioVIC™ Aerosol Collector, developed by MesoSystems Technology, Inc., serves as a front-end air sampler for biological detection systems. It is an impacter that preconcentrates the air stream, capturing large numbers of particles either into a small volume of liquid, into a small air stream, or onto a solid surface for delivery into the sensor. The BioVIC™ can be used with PCR, fluorescent-based optical sensors, mass spectrometry, pyrolysis GC mass spectrometry, or flow cytometry. Figure 5–5 shows a picture of the BioVIC™ Aerosol Collector.

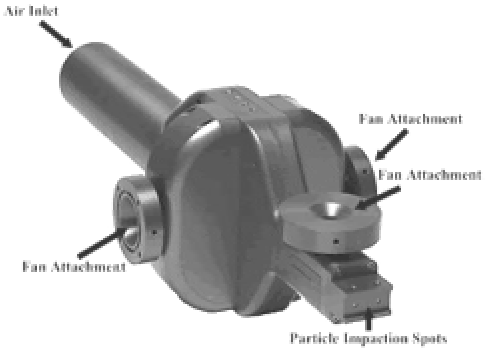

Figure 5-5. BioVIC™ Aerosol Collector, MesoSystems Technology, Inc.

5.1.2.3 Cyclone Samplers

A cyclone is an inertial device that is commonly used in industrial applications for removing particles from large airflows. A particle-laden air stream enters the cyclone body and forms an outer spiral moving downward towards the bottom of the cyclone. The larger particles are collected on the outer wall due to centrifugal force, and the smaller particles follow the airstream that forms the inner spiral and leave through the exit tube. Water spray applied to the outer walls of a cyclone facilitate particle collection and preservation. Several examples of cyclone samplers are discussed in the remainder of this section.

The Interim Biological Agent Detector System (IBADS) was initially developed for the Navy. It uses a wetted-wall cyclone to collect the aerosol particles into an aqueous sample. Variants of this device are in use in the Portal Shield Biological Detection System and in the current version of the Joint Biological Point Detection System (JBPDS). See figure 5–6 for an example of the JBPDS.

The Smart Air Sampler System (SASS 2000) is a device that has been independently developed by Research International and also uses wetted-wall cyclone technology. This hand-held device can operate on battery power. An example of the SASS 2000 is shown in Figure 5–7.

Figure 5-6. Joint Biological Point Detection System (JBPDS)

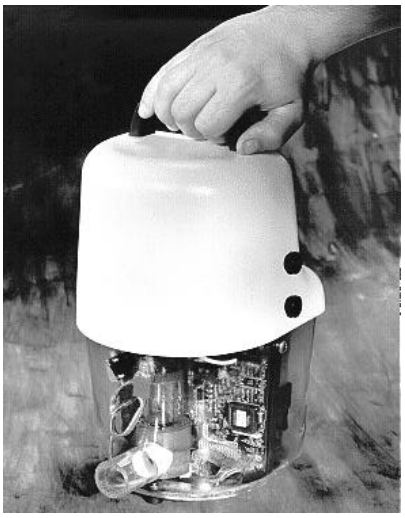

Figure 5-7. Smart Air Sampler System (SASS 2000), Research International

The Portable High-Throughput Liquid Aerosol Air Sampler System (PHTLAAS) is a small hand-held device that uses technology similar to the wetted-wall cyclone technology. This instrument concentrates the contaminants found in a large volume of air into a small volume of liquid for ultrasensitive semiquantitative detection. Zaromb Research Corporation has independently developed this device.

5.1.2.4 Hand-Held Sampling Kit

The Department of Defense Biological Sampling Kit (DoD BSK) is a prepackaged kit containing a panel of eight hand-held immunochromatographic assay (HHA) devices (i.e., able to simultaneously identify up to eight different biological agents), a dropper bottle of buffer solution, two sterile cotton-tipped swabs, and an instruction card. The DoD BSK is included in the sampler/collection section because it is used for field screening where the concentration of agent is expected to be high and not for positive identification. The kit is not to be used for screening soil samples since some soil constituents can cross-react with the HHA reagents if present in high enough concentrations. In addition, the DoD BSK should not be used for screening heavily dust-laden surfaces. Also, the kit is not sensitive enough to detect the minute amounts of precipitate that may fall out from an attack that originated from a distant location (e.g., a long line source release from several kilometers away).

The advantages of the DoD BSK are that it is inexpensive, reliable, easy to use, and the assays in the kit are improved concurrent with the assays in the other detection programs. Disadvantages of the DoD BSK are that it does not possess a generic detection capability (it is an identifier), and each kit is for one time use only.

5.1.2.5 Hand-Held Sampling Device

The BioCapture™ BT-500 Air Sampler was developed by MesoSystems Technology, Inc., and incorporates the BioVIC™ Aerosol Collector, also developed by MesoSystems Technology, Inc. It is a hand-held, battery-powered air sampler that collects airborne samples for quantifying concentration levels. The microbes are captured and concentrated into an aqueous sample for analysis by whole cell rapid detection, nucleic acid, or other liquid-based sensor systems. The removable single-use cartridge can also be archived for evidence of a biological incident. An example of the BioCapture™ BT-500 Air Sampler is shown in figure 5–8.

Figure 5-8. BioCapture™ BT-500 Air Sampler, MesoSystems Technology, Inc.

5.1.3 Detectors

Detectors are those components/instruments used to determine if the particulates are biological or inorganic in origin and if further analysis of the sample is needed. Some detectors require additional processing of a sample before it can be introduced into the detector, while others can use a sample directly from the environment. In this section, detectors are broadly divided into two groups, wet detection (flow cytometry) and dry detection (mass spectrometry).

5.1.3.1 Wet Detection (Flow Cytometry)

Cytometry is the measurement of both physical and chemical characteristics of cells. Flow cytometry (widely used as a wet detector for biological agents) uses the same technique as cytometry but makes the measurements of cells or other particles present in a moving fluid stream as they pass through a testing point. It measures particle sizes and counts particles in liquid suspensions through the use of laser light scattering. Flow cytometers involve sophisticated fluidics, laser optics, electronic detectors, analog to digital converters, and computers to provide an automated method for bio-chemical analysis and to process thousands of cells in a few seconds. Typically, the sample will also be treated by addition of a fluorescent dye that reacts with biological material (e.g., DNA). Flow cytometers have been commercially available since the early 1970s and increasingly have been used since then. Examples utilizing this technology are the Los Alamos National Laboratory Flow Cytometer (LANL) and the Becton Dickenson Flow Cytometer (FACSCaliber). They will be briefly discussed below.

The Los Alamos National Laboratory (LANL) Flow Cytometer employs a green (HeNe) laser diode. Particle size is measured by two light-scatter detectors, and fluorescence is measured by two photomultiplier tubes. This instrument is also known as the "Mini-Flow Cytometer" and is just 1.15 ft^3 in size, 30 lb in weight, and requires 1 kW of power.

The B-D Flow Cytometer FACSCount, manufactured by Becton Dickenson, employs a direct two-color immunogluorescence method and uses a green (HeNe) laser.

The B-D Flow Cytometer FACSCaliber, manufactured by Becton Dickenson, is a four-color Modular Analytical Flow Cytometer that uses a 15 mW air-cooled blue argon-ion laser and a red laser diode. The FACSCalibur also has an optional sorter. Figure 5–9 shows an example of the B-D Flow Cytometer FACSCaliber.