

REVIEW PAPER

Systems for Detection and Identification of Biological Aerosols

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ABSTRACT

Easy and inexpensive manufacturing of biological weapons, their complicated detection, expensive protection, difficult treating of affected individuals, selective impact only for people, animals or plants, are all factors making an effective defense against biological warfare agents very difficult. The aim of this study is an introduction to the systems for the detection and identification of biological aerosols containing dangerous bioagents. The basic techniques used for detection and identification of bioagents are described, including physical, molecular, immunochemical, and other ligand assays. Measuring systems and equipment for the individual techniques are summarised.

Keywords: Biological warfare agents, biological aerosols, detection system

NOMENCLATURE

BA	biological agents
BWA	biological warfare agents
DNA	Deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
LIDAR	Light detection and ranging
MALDI/TOF	Matrix-assisted laser desorption/ionization/ detector time-of-flight
PCR	Polymerase chain reaction
RT-PCR	Real time polymerase chain reaction

1. INTRODUCTION

History of use of biological warfare agents (BWA) outreaches 2500 years. Biological warfare agents have evolved from the plane use of cadavers to contamination of water resources through infected blankets to the development of specialized munitions for battlefield and covert use¹. Biological warfare agents otherwise biological weapons include microorganisms and biotoxins which are used to cause disease or death to humans, animals and plants. Biological warfare agents are capable infect many people and other living organisms in a large area with minimal economic costs and practically unnoticed. They can cause large-scale casualties and some of microorganisms are also available from natural sources (tularemia and anthrax). With the increased threat of terrorist attacks the misuse of BWA is a conceivable event. One of the problems is a misuse of biotechnology and food industry facilities for a simple and cheap production of the biological weapons.

One of the factors which makes effective protection against BWAs difficult is the complicated detection of their presence, expensive protection and only selective affection of living organisms. Initial symptoms after BWA

infection are difficult to distinguish from symptoms from common infections and medical treatments are ineffective.

There are several studies focused on military aspects as well as articles in specialized journals which provide comprehensive overview of various systems and devices for detection and identification of biological agents²⁻⁵. Furthermore, limited information about each of the detection systems are available from company literature, web pages and promotional items.

The most available systems are point detection devices, which are either in the stage of field testing or still on the laboratory level. Current biological agent detection systems are large, complex, expensive, and source of the false alarms. They can detect only a limited number of biological agents and are not able to identify the agents without direct contact. The comparison of the individual systems for attributes such a sensitivity, selectivity and resistance are not described anywhere. Only sometimes can be found articles describing experiments from field trials⁴, land applications of biosolids⁵ or describing natural sources of aerosols.

Biological detection technologies are still in immature stage of development comparing to the chemical detectors. From the analytical point of view can be distinguished detection-finding of undefined BWA inheritance in a sample, and identification-specific determination of BWA species based on characteristic molecular properties as DNA sequences, antigen markers, mass spectrum etc. Devices allowing only detection, give us information about contamination, but we don't know which type of BWA was used and what concentration is present in the environment. One example of this device is Stand-off Detection System. On the other hand, devices which gave us information about type of compound used are usually very complicated. Identification devices often need specially

trained operator, special environmental condition, etc. The majority of the identification devices are commonly found in the laboratories and mobile laboratories, but not practically used in the field conditions.

The equipment for detection and identification of biological agents can be also sorted based on different principles. One possibility is to divide devices based on their mobility and purpose: Handheld portable detection equipment, mobile laboratory detection equipment, screening devices, fixed-site detection systems, stand-off detection systems, biological samplers and biological reagent kits.

From other point of view the devices can be categorized based on the principle chosen for detection and identification: molecular recognition technologies (PCR, molecular hybrid and array techniques), immunological detection techniques (lateral flow immunochromatography, electrochemiluminescence, ELISA, time-resolved fluorescence, biosensors), physical techniques (flow cytometry, fiber optics, sample preparation, fluorescence, size and shape analysis, mass spectrometry, capillary electrophoresis, high performance liquid chromatography, flame spectrophotometry, gas chromatography), ligand-based techniques (Surface plasmon resonance), microscopy, standard culture and hybrid equipment.

2. BIOLOGICAL AGENTS

Biological agents are live pathogenic microorganisms and their toxic products, which are capable to cause mass infections or poisoning of people, animals and/or plants⁶. BAs can be sorted according to the causal agent as bacteria, viruses, rickettsiae and toxins. BAs used against humans are able to penetrate into the human body and cause disease^{7,11}.

The natural sources can be divided into several groups according to the occurrence: natural occurrence, agriculture and livestock production, the hospital environment and infectious diseases, industrial and other processes. Examples of the natural sources of bioaerosols include spores of plants, pathogens coming from bacteria and fungi. The natural and agricultural bioaerosols are typically blended together, for example inhalation of aerosol or dust during handling with the straw (hay), which may contain contamination from infected rodents⁶. In agriculture, a problems with mycotoxins produced during production and storage of grain may also be encountered. Transmission of disease through droplet infection is not only limited to the hospitals, but basically every day as just about the flu and other viral infections are found around. A more serious danger is the spread of bioaerosols in hospitals especially at the Department of Infectious Diseases, where is the risk of infection by different types of disease especially for patients with immunodepression and for exposed members of staff.

Also, industrial processes and landfills are powerful sources of bioaerosols consisting of pathogenic microorganisms, toxins, allergens, odors and dust particles. The mainly problem is a small distance between the site of an industry and residential areas. Most hazardous and unhealthy aerosols encountered in many productions are

not original biological aerosols.

Formerly BWAs were in the focus of researchers for their eventual use in armed conflicts, nowadays the possible abuse of BWA by terrorist groups is considered as the most dangerous threat. BAs are produced either as a dry powder or a liquid. The dry form can be stored longer and be dispersed more efficiently to the target, comparing to the liquid form which is easier to prepare for most of the agents. BWAs can be spread through air, water and food. We can found also dissemination of infected insects and rodents which came in direct contacts with the targeted individuals.

The most dangerous way of dissemination is dissemination in the aerosol form⁸. Bioaerosol can be artificially spread as practically all microorganisms and toxins including those which are impossible or difficult to spread in this form when occurring naturally. Considering a very difficult diagnosis, a different incubation period of the disease, wide area coverage by aerosol and current possibilities for traveling, the abuse of BWA in the form of a bioaerosol is the most anticipated. Comparison of lethal threat aerosol concentration and incubation period for specific toxins and bacteria^{9,10} in review² concerning with biosensor technology for detecting BWA, can find a table comparing different biosensors (analytes, detection limit, assay time etc.), for other detection methods, the comparison made in article current detection methods for biological agents of concern. The sources of aerosols include environmentally naturally present and man-made bioaerosols for testing or combat action.

Based on the way in which bioaerosols are disseminated during terrorist or military attack, it can be predicted how large area was affected. It includes linear and point sources. The linear source is represented by mobile equipment (aircraft or spraying car) although in this case based on to the intensity and wind direction, inversion, atmospheric pressure, characteristics of the agent itself it can involve a large area^{4,5}. The point source of contamination is typically immobile stationary equipment (nebulizer), which produces aerosols only around its area of placement.

Due to the complexity of the real environment, mathematical modeling including simulation studies also plays an essential role for the incidence of BWAs. Viability of microorganism is also necessary to know when doing identification of biological agents. The knowledge of the normal content of microorganisms in the monitored environment (a natural microbial background) is also important. For the classification of natural background, a four component system was proposed: (i) the potential pathogenicity for human, (ii) frequency, (iii) resistance to adverse environmental conditions, (iv) resistance to therapeutic agents (antibiotics). Overall hazards of the environment are thus based on combination of these four aspects⁹.

3. SAMPLING OF BIOAEROSOLS

The route of exposure is considered to be inhalation of aerosolized agents rather than skin exposure, contamination

of food or water or vector borne transmission, because aerosols are likely to be the quickest and most effective ways of exposing large numbers of people during the attack.

Many detection and identification devices are combined with a sampler. The detection system depends critically on samples collection and preparation, sampling strategies (where and how many) and the current state of collector and concentrator technologies. The simplest method of detection of microorganisms is draining of air through a suitable filter. Sampling can be a short or long term depending on type of system for detection of microorganisms. Sampling device can capture particles in a liquid or solid phase (agar), which is important for methods of analysis (microscopy, culture, bioassay, immunological assay etc.)^{11,12}. Factors affecting the recovery of microbes from air samples during subsequent analysis are sampling rate and time, organism or particles type, size and distribution, concentration factor, environmental factors (e.g. swirling winds), target detection method and choice of sampler (collection and recovery efficiency, loss of viability). During sampling of bioaerosols vitality maintain of the captured microorganism plays an important role.

For capturing bioaerosols particles, their surface charge is very important, too, as it can help to increase sampling efficiency. The charge is usually negative and can be used for preliminary distribution of captured particles. Detection of aerosol particles is possible even in the dry state using an electrostatic charge on the surface of detection¹³. Only three major sampler types for bioaerosols are available: Gravity devices, impactors and suction samplers.

Gravity methods are passive samplers which combine gravitational fallout and inertial processes to coated particles on microscope slide, agar plate, etc. They have a very limited use, because they are neither qualitatively nor quantitatively. Collection efficiency depends on particle size as well as air motion-turbulence, wind speed or shadowing. Some of types are: Vinzents sampler, wagner, and leith sampler.

Impaction samplers make use of accelerating air by rotating the collecting surface or using a fan. Impaction samplers are useful for collecting indoor bioaerosols including inertial impactors, impingers, cyclones, and centrifugal samplers.

Inertial impactors use constant wind speed, a fan or suction source for moving collection particles through sieves. Particles are usually deposited to solid or semi-solid surface and collection of particles allows size selective distribution. Some of types are: Sartorius MD8, Samplair MK2, Air ideal, Air samplair, and SAS.

Impingers use transfer of air through liquid (e.g. water, broth, mineral oil), where particles are removed from air to the liquid phases. This sampling system has some problems with pass through of particles, particle bounce, bubbling, evaporation of liquid and loss of viability. Some of types are: AGI, Biosampler by SKC.

Cyclones and centrifugal samplers use vortex creating sufficient inertia to trigger deposition of particles onto the

collection surface. Particles are intercepted for possible regeneration in liquid (cyclone) or in semisolid medium (centrifugal). Some of types are: SASS 2300 and 2400 by Research International, RCS Plus and High Flow.

Suction samplers include slit samplers, cascade impactors and filtration devices. Suction samplers can reclaim viable particles by direct impaction on culture media or by later culture of impinger fluid or filter eluates. Non-viable particles can often be identified by microscopic examination of slides.

Spore trap was initially designed for fungal spore and pollen. Particles are commonly captured on coated glass slide or adhesive tape. Advantages include properties as non-selective sampling, direct analysis after collection, disadvantages are masking problems and no assess of viability. Some of types are: Hirst, Burkhard, Air-o-cell, Allergenco.

Impactors are similar to spore traps, but the collecting medium lays on slide or agar plates. Some examples are: single stage or multistage (e.g. anderson), rotary arm samplers (e.g. rotorod, mesosystems BT550), sieve samplers and stacked sieves (e.g. SAS, Air samplair mas-100), Slit to agar samplers.

The principle of operation of a slit sampler is based on the passage of air by means of a vacuum source through the slit opening in the top of the sampler. The organisms in the air are impinged on the agar plate, which is rotated by means of a timer mechanism located in the base of the sampler. For example, the plate in a 2 h sampler makes one complete cycle in 2 h. Some of types are: Bio cassette by SKC, cassella slit to agar sampler, mattson garvin slit to agar sampler.

4. DETECTION SYSTEM AND EQUIPMENT

4.1 One Time Hand-held System

Simple hand-held systems are always based on lateral flow immunoassay technology for rapid field assay formats and clinical laboratory. These tests are usually single-use, inexpensive, with quick response (around 15 min) but they are designed to identify one agent per assay and they are not as sensitive as clinical laboratories. Lateral flow devices use immunochromatografic line assay or enzyme immunoassays forming different coloured product which can be read by human eyes or by an optical scanner. These systems look like as common home pregnancy tests. Lateral flow device have been developed by many companies especially for biohazard agents as ricin toxin, SEB and *botulinum toxin*, *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, *Brucella suis*, Variola virus. Although lateral flow device are accessible for use as simple test in the field and at first response there is little information available about their parameters except of company literature, web pages and broadsheets. There are many devices of different trademarks for example: ENVI Assay System Gold and FL (Envionics), SMART® (New Horizons Diagnostics Corporation), BioThreat Alert and Redline Alert (Tetracore), RAMP (Response Biomedical Corp.), Prime Alert™ Biodetection (GenPrime, Inc.),

NIDS® Handheld Bio-Threat Assay (ANP Technologies), BADD™ BioWarfare Agent Detection Devices (ADVNT Biotechnologie)¹⁴⁻¹⁷.

5. MOLECULAR RECOGNITION TECHNOLOGIES

5.1 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is one of the techniques, which are mostly used in the clinical laboratories for identification of microorganisms today. PCR enable exponentially amplify a single or a few copies of a piece of DNA set up thousands of copies of particular DNA sequence. Typical PCR relies on thermal cycling, consisting of cycles of repeated heating and cooling. Cycle, a series of 20-40 quick monitoring change of temperature, usually consisting of 2-3 discrete temperature steps, commonly three. Selection of parameter for each cycles used depends on the temperature, length of time, type of the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (T_m) of the primers.

Several small portable devices operate on this principle and are suitable for use in the field conditions. These devices differentiate from each other in number of individual samples which be analysed concurrently (four for HANAA, six for Bio-Seeq™ Plus, eight for RAPID) or in little bit different system of detection and speed of response. Bio-Seeq™ Plus^{2,18} made by Smith Detection uses LATE-PCR™ (Linear After the Exponential Polymerase Chain Reaction) technology. Ruggedised advanced pathogen identification (RAPID)^{2,3,19} made by Idaho Technology is build on platform of real time fluorescent PCR with accompanying agent specific freeze-dried reagents for the detection and identification of biological organisms. On the same principle RT-PCR exists another device called RAZOR (Idaho Technology)^{15,20} using cutting edge technology, which is designed for field use. HANAA is an acronym for the hand-held advanced nucleic acid analyzer developed by the Lawrence Livermore National Laboratory in 1999, which analyzes biological samples for the presence of specific DNA sequences by application of Taqman fluorphors introduced into the sample before it is inserted into the heater chamber.

There are some other devices using different kinds of PCR such as: SmartCycler®21 or GeneXpert System (both by Cepheid), COBAS AMPLICOR™ Analyzer or LightCycler™ (by Roche Applied Science), which can be used in mobile laboratory or as a part of bigger system.

6. ELISA

The method has several variations, all of which are based on the highly specific interaction between antigen and antibody. The principle of this method is based on competition antigen deposited on a solid surface and antigen in the sample. Boths antigens competed on a limited number of binding sites on the antibody. The more antigen analyzed sample contains, the less antibody will bind to the anchored antigen. Unbound substances

are removed and subsequently added an enzyme labeled secondary antibody against bound antibody. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

The ArrayTube (AT)^{22,23} chip by Clondia combines a microtube-integrated protein chip with the classical procedure of a sandwich-enzyme-linked immunosorbent assay. The specific interaction is visualized by applying the colorimetric staining technology by using horseradish peroxidase (HRP) molecules conjugated to target molecules, which bind to the AT-array and catalyze the local precipitation reaction of tetramethylbenzidine (TMB). The resulting precipitation pattern images are detected with the ArrayTube reader.

6.1 Biosensor/Immunosensor

Biosensor is an analytical device which combine a biological component with physico-chemical detection of signal (such as luminescence, electrochemical or optical signal). Many different types of antibody-based sensors have been developed. Immunosensors offer a highly specific probe technology due to the fact that antibodies recognize very specific sites or cellular components. Alternatively, the binding can be monitored in a sandwich assay in which a second antibody labeled with a fluorescent dye binds to another epitope on the captured cell or to the probe antibody.

Problems for measurements with biosensors/immunosensors include nonspecific binding, degradation of the antibodies over time, reproducibility of the antibodies and a problem with cross-reactivity. It means that closely related organisms cannot be frequently distinguished by immunochemical techniques.

Array biosensor (Naval Research Laboratory)²⁴ uses a sandwich assay for detection of biological agents which are detected by a CCD camera. This device can simultaneously detect and identify different targets on the same slide, if multiple 'capture' or 'tracer' antibodies for different BW agent are used. The responses are fast (10-15 min), sensitive, and specific.

The Biosensor 2200R from MSA25 is working on principle of an immunoassay detection technology, when the sample is mixed with magnetic particles and the generated sandwich complex is fluorescently labeled. Intensity of the fluorescence is measured after the capturing of magnetic particles in the sensitive area. The speed of measurement is 5 min time-to-answer.

The bio detector²⁶ licensed to Smiths Detection-Edgewood, Inc., by molecular device corporation can simultaneously detect up to eight different agents using immuno-ligand assay chemistries with a light-addressable potentiometric sensor (LAPS). During the immunospecific reaction, biotin and fluorescein-labeled antibody are used in solutions together with anti-fluorescein antibody, conjugated to the enzyme urease. In detection phase substrate the enzyme reaction of urease provides change of pH which is detect by the LAPS transducer.

The smartbio sensor (SBS)¹⁵ by smiths detection uses an 8 sensor array and samples are stored for possible

further analysis. The RAPTOR (research international, inc) is a portable, 4-channel automatized fluoroimmunoassay system for the rapid detection of protein toxin, viruses, and bacteria^{27,28}. The Analyte 2000 (research international, inc. in conjunction with the naval research laboratory) is a 4-channel, single wavelength fluorometer using evanescent-wave fluoroimmunoassays for detection biochemical species.

Fluorescent aerodynamic particle sizer (FLAPS)^{29,30,35} simultaneously measures each individual airborne particle, the scattered-light intensity and the fluorescence emissions in two distinct wavelength bands by two highly-sensitive photomultiplier tubes. A variation of the FLAPS particle sizer is the ultra violet aerodynamic particle sizer (UV-APS)³¹ which 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 single-particle fluorescence counter (SPFC)²¹, developed by the naval research laboratory (NRL) measures intensity of UV fluorescence from the particles following excitation with a 266 nm wavelength laser pulse. VeroTect (Biral)³² combines the proven ASAS technology of aerosol size and shape characterization with technology for generic characterization by particle fluorescence using 280 nm excitation wavelength. This combination of light induced fluorescence, together with particle size and shape information allows VeroTect to characterize the aerosol to an extent that is not currently achievable by any other sensor.

7. PHYSICAL TECHNIQUES

7.1 Fluorescence Methods

Fluorescence approaches involve excitation of molecular components of a biomaterial with light, usually in the ultraviolet (UV) region of the spectrum. The excited component spontaneously reverts to an unexcited state followed by emission of light at different wavelengths. Individual devices differentiate in source of light, excitation wavelength, numbers of measuring channels, etc.

7.2 Capillary Electrophoresis

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent on the charge of the molecule, the viscosity, and the atom's radius. Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation. One item that utilizes this technology is the agilent 2100 bioanalyzer from agilent technologies. Application capillary electrophoresis for the detection and identification of biological aerosols will be mainly in its combination with other techniques, particularly mass spectrometry.

7.3 Flow Cytometry

Flow cytometry provides the differentiation and

determination of the exact number of the cells contained in the suspension sample on the basis of their different optical properties. Method allows simultaneous measurement and analysis of physical-chemical characteristics of cells or other biological particles during their passage through the laser beam. At the moment when the beam crosses the cell leads to refraction and scattering of light, which according to the direction and angle of refraction is known as direct scattering-forward scatter (FSC) and side scatter-side scatter (SSC). FSC correlates with the cell volume and SSC depends on the inner complexity of the particle. Besides the parameters of refraction and dispersion of light is also detected by fluorescence passing through the cells or particles. Fluorescent dyes (fluorochromes) linked to the analyzed cells or particles absorb light of a specific wavelength emitted by the laser and then radiate (emit) part follows absorbed light but has a different wavelength. In flow cytometry fluorochromes are used, which generally have the same absorption spectrum, but other emission. Cells and cell-free structures (bacteria, viruses) do not contain fluorescent pigments, but they can be labeled with fluorescent substrates, which often bind to their DNA.

MICROCYTE® Field (by BioDETECT AS)²¹ is commercially available rugged, portable flow cytometer for on-site verification of the potentially contaminated sources or suspicious materials. It can be also used for on-site analysis of microorganisms. Data are presented as 2-color histogram or as 2-conditional dot plot.

7.4 Optical

Stand-off detection system called light detection and ranging (LIDAR) are designed for detection and identification of biological agents in large distances, before the agents get to other types of detectors. There is no sampling method, but laser is employed for the detection. Short laser pulses are passing through the atmosphere and are partially reflected back from the particles in the atmosphere as molecules, aerosols, clouds and dust. These systems are able to identify small particles on the distance of 30 km - 50 km, but they do not distinguish between the type of particles in the atmosphere, i.e. whether it is the BWA or not.

In general all the system provide the cloud configuration (size, shape, and relative intensity) and cloud location (range, width, height, above ground and drift rate), but they do not identify biological material. There are some devices available as laser-induced-fluorescence sensor the biological agent warning sensor (BAWS)⁸ by lockheed martin, a prototype SR-biospectra³³, long range biological stand-off detection system (LR-BSDS).

The BAWS sensor is a local or point sensor because detects only particles that actually flow through this sensor. The BAWS can be consider as short-range lidar, where the distance from the laser transmitter to the sensed bioparticles is extremely short-only a few centimeters. Biospectra is also a short-range compact spectrometric lidar for detect of bio-threats in aerosols over critical area.

7.5 Spectroscopy

Biological alarm monitor (MAB)²¹ by Proengin USA is a biological alarm monitor working on a principle of flame spectroscopy. It continuously analyses particles in the atmosphere and searches for specific chemical structures of bacteria and toxins and gives alarm when the concentration of these suspects particles in the air increases. It allows triggering a bio sampler to determine the pathogenic characteristics of the detected particles. In 2009 Proengin and Bertin technologies have created a new concept of three step biological detection system consisting of MAB, coriolis (bio-air sampler for airborne pathogens detection) and the KIM (field biological identifier) which has been recently adopted by french forces for overseas missions. The complete system allows receiving an answer about the level of danger level within 20 minutes after its first detection.

The chemical biological mass spectrometer block II^{21,34} by the oak ridge national laboratory and their partner, orbital sciences corporation is system for the detection and identification of chemical and biological warfare agents designed for use in reconnaissance vehicles and other mobile detection systems. The CBMS block II consists of a mass spectrometer module, sample introduction module, biosampler module, and a soldier display unit. The biodetection system is based on direct sampling and thermolysis/derivatization of biological particles. As a 'dry' system it minimizes the operating costs.

The software tool bio profiler by bruker daltonics is used for identification of microorganism based on their protein fingerprint in the MALDI-TOF mass spectrum by comparing their individual peak lists with the available database or your own database.

Ultraviolet aerodynamic particle sizer® (UV-APSTM)³⁵ spectrometer which measures the concentration and aerodynamic diameter of particles in the size range of 1 µm – 20 µm by light scattering and time-of-flight measurements, complemented by the measurement of fluorescence emission (420 nm – 575 nm) after excitation by a pulsed 355 nm laser. The UV-APS spectrometer was developed originally to detect the presence of biological agents.

Fourier-transform infrared spectroscopy (FTIR) can be described as passive optical technique, which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. As can be seen on data³⁶, good application identification algorithm gave successfully discriminated between the BG material and the kaolin dust. The results of this study suggest practicability of detecting biological aerosols with passive FTIR sensors.

7.6 Integrated Detection System

Integrated detection systems usually consist of several components. Detection devices have relatively low specificity, but a high response speed. They usually work on the physical

principle, which continuously monitors the concentration profile and structure of particles in the monitored environment. Results of the measurements of the sample are compared with the results of the previous and in the case of significant differences or finding the data profiles appropriate for known biological warfare agents, this system can start up other activities -either the alarm or immediately start the sampling apparatus and confirm the result by a suitable identification method.

The biobriefcase³⁷ (Lawrence Livermore National Laboratory) is an integrated, miniaturized aerosol collection and analysis system. The device consists of three components: the aerosol collector module, the PCR module, and the immunoassay module. Capillary electrophoresis is use as a detector. The PCR module autonomously conducts DNA purification, concentration and amplification. System can operate autonomously as long as 30 days without maintenance, so it can be used for passive environmental monitoring, or the aerosol collector for field laboratory operation.

The interim biological agent detector (IBAD)¹⁵ is a simple semi-automated point detector system for using on a shipboard. It consists of a particle sizer/counter, particle wet cyclone sampler and a manual hand held immunochromatographic assay (HHA) identifier.

Biological integrated detection system (BIDS²¹) consists of 5 major sub-components: (1) vehicle, (2) shelter, (3) auxiliary equipment, (4) power and (5) biological detection suite. Test equipment are high volume aerodynamic particle sizer (HVAPS) by TSI inc., liquid sampler (LS), biological sampler or single liquid sample collector, flow cytometer (FCM) by coulter corporation, threshold workstation (THS - is a registered trademark of molecular devices corp.) and adding biological detector, mass spectrometry³⁸. BIDS integrates aerodynamic particle sizing, luminescence, fluorescence, flow cytometry, mass spectrometry and immunoassay technologies in a hierarchical, layered manner to increase detection confidence and system reliability due to the fact that it can detect all types of biological agents and identifies specific agents of interest.

Joint biological point detection system (JBPDS)²¹ is a fully automated biological detection and identification equipment which it can be integrated into multiple platforms (vehicles, ships, trailers) or in a stand-alone configuration. It consists of a detector, collector, fluid control system and identifier. The identifier includes equipment which contains antibodies of specific biological warfare agent antigens. JBPDS provides the possibility to collect and preserve samples for next confirmatory analyses.

The 4WARN vital point bio-sentry system by general dynamics, canada is a third generation of fully-automated biological agent detection and identification system. System has a flexible design so that it can be received in various configurations and module combinations according to specific mission scenarios or vehicular/naval platform. For the generic detection system, fluorescent particle detection (FPD) and biological real time sensor (BARTS) are used. Specific agent detection is based on

antibody assay strips and automated reader or multi-site polymerase chain reaction (PCR). System is coupled with a liquid sample collection module, a battery module and a processor control module³⁹.

Autonomous pathogen detection system^{40,41} (APDS), was developed at Lawrence Livermore National Laboratory for continuous sampling of the air for airborne pathogens and toxins. The system can run unattended for 1 week. The APDS uses capture beads coated with antibodies specific to the target pathogen. It consists of an aerosol collector, a sample preparation subsystem and two subsystems for detecting and analyzing of the samples: one based on PCR and the other based on flow cytometry, which uses antibodies for identification of pathogens.

The Biohawk® by Research International⁴², is a portable 8-channel bioassay system integrated with an aerosol collector. Bioassays are performed within a small disposable credit card-sized plastic assay coupon where all target-specific reagents are incorporated. Detection uses 'sandwich' assay protocol with antibodies to bind targeted pathogens to the waveguide surfaces, and fluorophore-tagged secondary antibodies to create a fluorescent signal when the waveguides are irradiated with 635 nm solid state laser light.

Short comparison of basic characteristic (e.g.: principle of detection, limit of detection, application, stage) for measuring systems and equipment is included in Table 1. Table 2 was containing a compare of limit of detection for selected measuring systems for specific toxins and bacteria.

8. CONCLUSIONS

The aim of this review was overview of methods and equipment available for detection and identification of biological warfare agents, especially focused on the bioaerosols. Many factory-made devices are commonly used at potentially hazardous places as airports, subways, stadiums, state buildings, semi-enclosed facilities. These devices are also usually used for military functions. Devices mentioned in sections Integrated detection system and spectroscopy and optical, are in most cases used for these purposes. Some of the devices from the other sections are mainly used in laboratories or as fast screening tests (one hand held systems). Information about each of the detection systems and its applications, are available from company literature, web pages and promotional items. The progress goes in the direction of more efficient and user friendly systems, probably in the way of miniaturization and simplification. Except for the one hand held systems, other devices need specially trained operator. Detection and identification devices commonly need (specifically bioaerosol) some sampling devices. This is the case of big complex systems as 4WARN, but not for example BIOSENSOR 2200R. Detection or identification of one sample is not only detection/identification but also sampling, concentration etc. takes some time.

Future progress in detection of biological weapons will be associated with increasing risks of bio-terrorism.

It is likely that importance of field analysis will increase in way of simply usable devices in the biological protection system. It is hard to predict how quickly it evolves into new trends for rapid detection and identification of biological agents. It is supposed that the whole detection area will focus on the potential misuse of biologist substances including their genetically or otherwise modified variants.

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