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Identification of biological warfare agents – recent progress and future trends

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English summary

This report provides a brief summary of current and developing technologies available for identification of biological threat agents. In addition, some representative examples of bio-identification platforms are given. Discussions regarding the future trends and prospects in the field of bio-identification technologies are included.

The responsible nation for writing this report was Finland with assistance from the other nations. The Finnish report is enclosed to this FFI report and starts at page 5.

Sammendrag

Denne rapporten er en leveranse i NORDEFCO-samarbeidet mellom Norge, Sverige og Finland med tittelen "The biological identification and detection capabilities (BIDC)" (05.06.2015). Hovedfokuset i leveransen er å beskrive hvordan dagens teknologiske tilnærminger og instrumentering kan brukes til å identifisere biologiske trusselstoffer i en kjemisk, biologisk, radiologisk og nukleær (CBRN) sikkerhets- og forsvarssammenheng.

Rapporten beskriver metoder og teknologier for identifikasjon av biologiske trusselstoffer. Den inneholder en kort oppsummering av teknologier som per i dag er tilgjengelige for identifikasjon av biologiske trusselstoffer, inkludert en diskusjon av fremtidige trender innenfor dette området.

Finland har hatt hovedansvaret for å skrive denne rapporten med innspill fra de andre nasjonene. Rapporten er i sin helhet vedlagt og starter på side 5.

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Preface

This report is a part of the NORDEFCO subgroup Biological Identification and Detection Capabilities (BIDC) (05.06.2015). The subgroup investigated the possibilities for co-operation within biological stand-off and point detection and identification, in order to improve biological defence. The aim of work was cost savings for the participating nations and enhanced capabilities. This is achieved by means of information exchange as well as identifying areas of common goals, needs, capabilities and harmonization. The objective is to seek possibilities to common procurement, education and training, standards, testing, burden sharing, resource optimisation and interoperability.

This report is a continuation of our previous paper "Detection of Airborne Biological Agents: Technological review and outlook. The scope of this paper is briefly to describe the current and developing technologies for identification of biological threat agents. In addition, a few examples of identification components and platforms will be given.

This report is aimed for military decision makers to aid in establishing and maintaining Nordic co-operation. The main focus of the report is describing current technological approaches and instrumentation being used for the identification of deliberate release biological agents in chemical, biological, radiological or nuclear (CBRN) security and/or -defence context.

1 Background and definition

Biological warfare agents (BWA) are harmful pathogenic microbes or toxins which are being used as a weapon to cause disease or death on target population. Although a large number of pathogens or toxins are suitable for BWA only twelve of those have been weaponized [1]. Today the use of BWAs as a means of warfare is extremely low. Weaponized BWAs are usually regarded as weapons of mass destruction or casualties (WMD). In the context of bioterrorism and BWAs as means of asymmetric warfare implication are often limited. Contrary to public believe establishment of offensive biological weapon capability is not plain and simple. There are many obstacles i.e. technological, political and economic. Modern microbiology and biotechnology have significantly advanced on the 20th century creating huge opportunities to develop highly sophisticated BWAs.

Nevertheless said before, biological warfare agents have remained a persistent concern for the people responsible the national security.

Besides prospects for BW weapons development modern technology provides tools to improve biodefence capabilities. Development of detection and identification methods for BWAs is an important component in our preparedness to protect troops and public health from the harmful effect of biological threat agents.

2 Units of measure for biological warfare agents

In microbiology colony-forming unit or CFU is gold standard to estimate the number of viable bacterial and fungal cells in a sample. In virology the counterpart of CFU is PFU or plague-forming unit. In the case of biotoxin mass based units such as µg or µmoles are being used. Among biodefence community for aerosol samples Agent Containing Particle Per Liter Air (ACPLA) is often the unit of choice [2], [3] to describe the number of particles contained in the air. It can be easy to measure for biological agents like bacterial spores with the use of slit-to-agar samplers. Measurement of ACPLA is robust and simple to perform and has been widely used, especially in connection of testing of bioaerosol detectors.

Unfortunately it provides little useful information about the nature of the bioaerosol particles. ACPLA doesn't reveal how many active microbial cells are present. A single particle or ACPLA may contain one or tens even hundreds of agents (Figure 2.1).

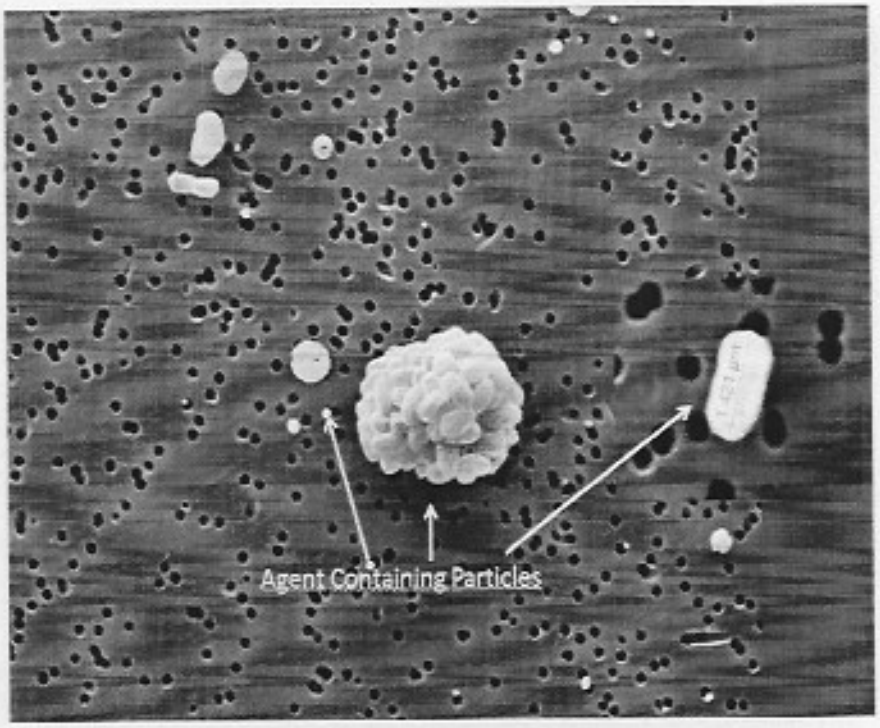


Figure 2.1 SEM image of aerosolized Bacillus atrophaes spores collected on polycarbonate filter. Spores are generated by nebulizing Ba suspension (10^8 spores/ml). Image Sirpa Mustalahti DFRA.

Moreover ACPLA particle size distribution remains unknown. It's well recognized that only bioparticles having particle diameter 1-5 µm are dangerous. Thus, to evaluate the health hazard from deliberate release of BWA the ACPLA unit is not useful. National Research Council in USA has suggested a new unit for the hazard prediction. The proposed hazard unit is Biologically Active Unit Per Liter Air or BAULADae, where Dae is the particle aerodynamic size. It measures concentration as a function of aerodynamic particle size. To be biologically active, it necessitates the presence of an active agent (infectious microbe or toxin molecule) in a particle. Aerodynamic particle size gives a rough estimate of the number of active agents in a particle. BAULADae provides useful information needed to estimate health hazard. However the proposed BAULADae is more complex to determine and for instance requires active particle counting as a function of aerodynamic particle size. In practice the determination of BAULADae is a challenging task. Biological activity of most aerosolized BWAs is not known and the methods for determing of agent characteristic such as viability, culturability, virulence etc. are challenging to measure. Moreover, the PCR based method currently being used for bio-identification cannot discriminate active agents form inactive agents. A new unit of measure ie "Total Agent per Liter Air with particle size distribution" or TALAp has been suggested [4]. It contains the two most important variables for evaluation of biodetectors and identifiers. The use TALAp provides significant improvements over ACPLA because it allows measurement of the particle size distribution and the amount of agent present.

A rough estimation of viable agents in ACPLA can be done by measuring concurrently aerosol concentration with the slit-to-agar sampler and air-to-liquid samples. In an aerosolization experiment the one ACPLA particle was found to contain three viable *Bacillus atrophaeus* spores (see Figure 2.2) [5].

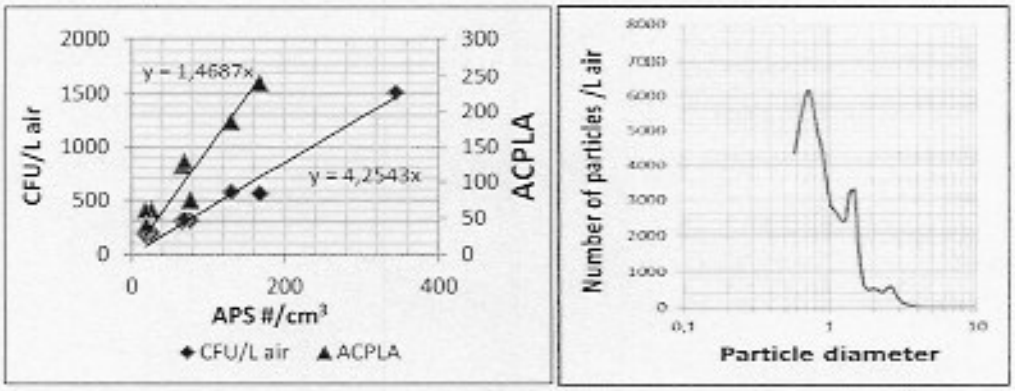


Figure 2.2 Correlation between total aerosol count (particles/cm³ as counted by an Aerodynamic Particle Sizer TSI APS) and viable particle count. One APCLA particle contains three viable spores. This is based on assumption that air-to-liquid-sampling (SKC BioSampler®) disperses any spore-to-spores agglomerates into single spores and collection efficacy of both sampling techniques are equal. Graph on the right shows the particle size distribution in the aerosol cloud generated by nebulizing from Bacillus atropheaus spore suspension. Probably, peaks at 1.5 and 2.4 µm represent spore-to-spore agglomerates.

3 Evaluation of health risk from bioaerosol exposure

The health risk from deliberate release is a com-'-- issue as shown in Figure 3.1. Risk assessment from bioaerosol exposure is out of the scope of this paper. However it would be worthwhile to discuss to discuss it to some extent. In recent study National Research Council has presented a framework for evaluating total health risk posed by bioaerosol exposure. The health risk consists of two components: health hazard posed by an agent and the physiological response of the individual exposed.

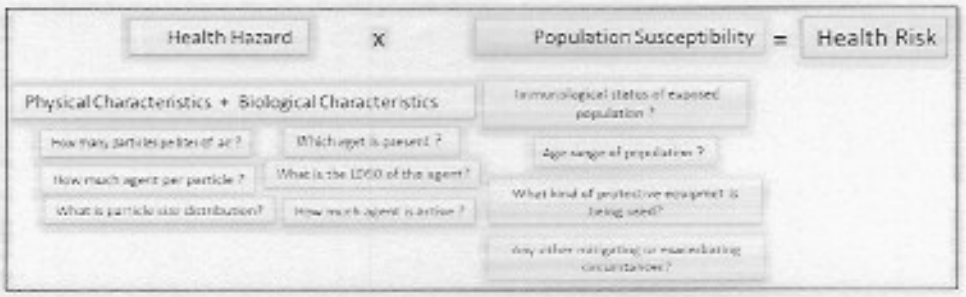


Figure 3.1 A framework for evaluating the health risk posed by aerosolized biological warfare agent exposure [6].