A Framework for Assessing the Health Hazard Posed by Bioaerosols

Committee on Determining a Standard Unit of Measure for Biological Aerosols, National Research Council

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Committee on Determining a Standard Unit of Measure for Biological Aerosols
Board on Chemical Sciences and Technology
Board on Life Sciences
Division on Earth and Life Studies

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Preface

The Committee on Determining a Standard of Measure for Biological Aerosols was convened by The National Academies in response to a request from the U.S. Department of Defense (DOD), Joint Program Executive Office for Chemical and Biological Defense (JPEO CBD), Joint Project Manager, Nuclear, Biological and Chemical Contamination Avoidance (JPM NBCCA), Product Director, Test Equipment, Strategy and Support (PD TESS).[1] As the committee title indicates, PD TESS requested the evaluation of current units of measure of biological aerosols and, if necessary, the development of a new standard unit of measure to aid in the development and testing of biological warfare agent detectors. Specifically, an evaluation of the continued use of Agent-Containing Particles per Liter of Air (ACPLA) in test and evaluation of detectors was requested. The full statement of task for the committee can be found in Appendix B.

In approaching the statement of task, the committee requested input from representatives of the Joint Program Manager for Biological Defense; the Joint Program Manager for Nuclear, Biological, and Chemical Contamination Avoidance; the Joint Requirements Office (JRO); Dugway Proving Ground (DPG); and the United States Army Medical Research Institute for Infectious Diseases (USAMRIID). These speakers brought important, complementary perspectives to the question, and gave the committee useful insights on the impact a new unit of measure would have on the testing and evaluation community. These presentations and discussions were instrumental in guiding the committee to the development of the framework for evaluating health risk described in the report. We believe the recommendations will strengthen the existing testing and evaluation community by creating a single unit based on the specific health risks presented by different biological agents.

The committee met three times in person in Washington, DC, and numerous times by teleconference over the course of eight months. The timeline and workload required considerable attention from the committee, and this report would not have been possible without their consistent dedication and patience. Throughout the project, the staff of the sponsoring office, PD TESS, provided considerable support and interest in the study. The committee sincerely appreciated their willingness to answer questions and provide necessary background information.

J. Patrick Fitch, *Chair*

[1] The abbreviation PD TESS will be used to indicate this organization.

Acknowledgment of Reviewers

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published report as sound as possible and to ensure that it meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Mark Buttner, University of Nevada, Las Vegas
Richard Chang, Yale University
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Janet Macher, California Department of Health Services
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D. Warner North, NorthWorks, Inc.
Donald Prosnitz, Lawrence Livermore National Laboratory
Edwin P. Przybylowicz, Eastman Kodak Company (retired)
Chad Roy, Tulane National Primate Research Center

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Dr. David Franz, Midwest Research Institute. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Summary

INTRODUCTION

Biological warfare agent (BWA) detectors are designed to provide alerts to military personnel of the presence of dangerous biological agents. Detecting such agents promptly makes it possible to minimize contamination and personnel exposure and initiate early treatment. It is also important, though, that detectors not raise an alarm when the situation does not warrant it. Such "false positives" may result in unnecessary precautionary measures and could impact a mission's success. For biological agents, establishing an appropriate level of sensitivity for an alarm is especially difficult because biological agents are diverse and vary widely in infectivity and virulence. Thus, detectors ideally should be capable of discriminating between highly infectious or virulent agents—to which they must be very sensitive—and innocuous ambient environmental microorganisms or low-risk ones—for which an alarm would be undesirable.

It is a challenge, then, to choose what physical characteristic detectors should measure, since the effect of interest is inherently biological activity. Ideally, a single characteristic, common to all hazardous agents, and accurately quantifiable, would be most useful. Such a characteristic would make it straightforward for a test and evaluation program to compare the accuracy and sensitivity of different detectors. The U.S. Department of Defense (DOD); Joint Program Executive Office for Chemical and Biological Defense (JPEO CBD); Joint Project Manager, Nuclear, Biological and Chemical Contamination Avoidance (JPM NBCCA); Product Director, Test Equipment, Strategy and Support (PD TESS)[1] sought a standard unit of measure that could be used for biological material independent of the state of the material and independent of agent type. PD TESS asked the National Academies to conduct this study, addressing the use of measurement in the test and evaluation of aerosol detectors and evaluating whether the standard unit currently in use, Agent-Containing Particles per Liter of Air (ACPLA,) is the most appropriate measure and what alternatives exist. As the name suggests, ACPLA is a measure of the number of particles in a liter of air that contain a viable biological agent. Formally, the term applies to bacteria, but it can be modified to refer to viruses or toxin molecules. The question considered in this report is whether ACPLA is an appropriate unit of measure for use in the evaluation of aerosol detectors and whether a better, alternative measure can be developed.

This report discusses several problems with the use of ACPLA. First, ACPLA does not distinguish between "active" and "inactive" biological agents. (While the term "active" can have different meanings, for the purpose of this report the term is used as shorthand for the form of the biological agent that can cause harm—live bacteria, infective viruses and active biotoxins. The term is more general than "viable", which is specific to bacteria and refers to the capacity to grow in culture, as opposed to the ability to cause harm.) Second, ACPLA does not distinguish between virulent and innocuous strains of bioagents. Third, even as a simple measure of quantity, ACPLA falls short because it does not distinguish between particles containing a single

[1] The abbreviation PD TESS will be used to indicate this organization.

organism or molecule and those that might contain hundreds or thousands of units of the bioagent. Finally, the health effects of particles containing bioagents can be dramatically affected by the size of the particle—small particles can be drawn deeply into the lungs where they are more harmful, while large particles may only reach the nasal passages, or not be inhaled at all. ACPLA makes no particle size distinction.

The problem with a unit of measure that does not include such parameters can be illustrated by imagining the response to two different threats of a detector measuring ACPLA: in one scenario, an average liter of air contains 100 particles, each carrying one avirulent bacterium. The detector would give a reading of 100 ACPLA. In the other scenario, an average liter of air contains 1 particle carrying 100 extremely virulent, live bacteria. The detector would need to be sensitive down to the level of 1 ACPLA to detect this attack. If the detector were set to sound an alarm at, say, 10 ACPLA, the detector would alert military personnel to the first attack—which is, in fact, harmless—and fail to alert to the second attack—which might be extremely dangerous. Even if the detector were sensitive enough to detect 1 ACPLA, it would still sound an alarm in both situations, where only one warrants taking precautionary measures.

The challenges described above are just some of those associated with the measurement of biological species. The complexity of the bioagent threat is such that there is only one relevant characteristic shared by all agents of interest: the capacity to interact with the human body and potentially cause harm. Thus, a unit of measure that considers the health hazard posed by a given concentration of aerosolized biological agent in air would allow comparison across all agent types against a characteristic that would have real utility both for test and evaluation of detectors and in the field.

ACPLA is easily understandable and measurable; thus, it is straightforwardly incorporated into a system of detector requirements and evaluation, but it fares poorly in the more complicated task of providing a tool to measure the actual hazard posed by a biological attack or a system's ability to detect that hazard. ACPLA focuses attention on a generic characteristic (quantity of agent containing particles) that cannot be related, even relatively, to health hazard. Instead, it would be more useful to adopt a framework of measurement that makes it possible to evaluate relative hazard by including agent identity and activity, particle size and infectious dose. The new measurement framework would be more complicated than ACPLA. Not all of the information needed to compare the health hazard of different agents is readily available. Even with imperfect knowledge, however, the new framework could be implemented with current technology. More importantly, implementing the new framework would serve to focus future development efforts on detectors that measure parameters relevant to health risk in both military and civilian settings.

CHARGE TO THE COMMITTEE

At the request of PD TESS, the National Research Council was asked to evaluate current units of measure for biological aerosols and, if necessary, determine a standard unit of measure that can be used for biological material independent of the state of the material (aerosol or aerosol resuspended in liquid) and independent of agent type (bacteria, viruses, or toxins).

The committee addressed the following questions:

- Is there a single unit of measure that is appropriate for use in the evaluation of aerosol detectors?

- What are the possible alternatives to the use of ACPLA and what are the advantages and disadvantages of their use?
- Are different measures appropriate in different circumstances?
- Is there a robust way to convert between various units of measure?

RESPONSE TO THE CHARGE

Evaluation of existing units

Some of the limitations ACPLA were discussed above. Even the real advantages of ACPLA—its relative simplicity and clear quantifiability—are compromised by the fact that, in practice, ACPLA is measured in many different ways. Some instruments may determine the identity and amount of live agent by culturing concentrated air samples on solid agar medium. Other instruments measure such characteristics as the particle count or sample fluorescence. ACPLA does not explicitly make a distinction between active and inactive agent, and instruments that measure different characteristics may provide different ACPLA assessments of the same sample. For example, an instrument that counts colony-forming units (CFUs) would be measuring live bacteria, while an instrument that counts agent genome equivalents using polymerase chain reaction (PCR) would be measuring both live and dead bacteria. Each could be used to infer the number of agent-containing particles, but they could give different measures of concentration. Finally, because ACPLA does not account for the dramatic differences in the health risks posed by different agents, the recipient of an ACPLA count must know what agent is detected, and understand the health risks posed by the specific strain in order to determine the proper course of action. Therefore, ACPLA alone does not provide enough information to determine the truly relevant information—whether a health threat exists.

Another possible unit, agent concentration in irreducible units (e.g., spores), suffers from similar issues as ACPLA. This unit, unlike ACPLA, does reflect the total amount of agent present, but like ACPLA, fails to measure activity or particle size, which can significantly change both the lethality and the infectivity of the attack.

Another alternative measure is the number of colony-forming units (CFU) of bacteria, plaque-forming units (PFU) of virus, or mass of toxins per unit volume of air. These measures also lack the detail needed to fully assess hazard. Important factors, such as particle size, are again lost with these units. These measurements, in the case of bacteria and viruses, do provide useful information on biological activity—a necessary condition to determine whether an agent is capable of producing an adverse biological outcome—but they reflect fundamental biological differences of the different classes of agents and are not directly interconvertible.

After a survey of the literature to identify units describing the concentration of biological aerosols, the committee concluded that no single unit of measure is available that could be used directly to compare the health hazard posed by different biological agents and the many forms in which they might be encountered in an attack.

An integrated framework that allows comparison of *hazard*

The committee concluded that in order to be useful and comparable across all biological agents and detection systems, measurements of biological agents must ultimately be related to health hazard. The capacity to cause harm is a quality shared by all pathogens, including those not associated with biological warfare, such as SARS, avian influenza, or the toxins associated with red tide. While this report focuses on biological warfare agents, the concepts presented can be applied to a broad range of airborne pathogens and biological toxins.

Two critical factors determine the probability that a BWA aerosol exposure will produce an adverse health outcome—the *hazard* posed by the agent and the *physiological responses* of the individuals exposed to it. A cloud of smallpox virus is a *hazard*, but it may pose no health *risk* if all of the exposed individuals are effectively immunized against smallpox. The hazard posed by an agent is determined by its physical and biological characteristics: identity and strain of agent, activity, virulence, and particle size.

For the purpose of developing, testing and evaluating bioaerosol detectors, it is the *hazard* that would be measured. This report proposes a unit—Biologically Active Units/Liter of Air or $BAULA_{Dae}$— that quantifies the hazard posed by a particular bioaerosol where D_{ae} is the particle aerodynamic diameter. The $BAULA_{Dae}$ unit can then be embedded in a full health risk framework that places that hazard in the context of the likely physiological impact on the particular population in question. The parameters that are included in $BAULA_{Dae}$ are summarized in Figure S.1.

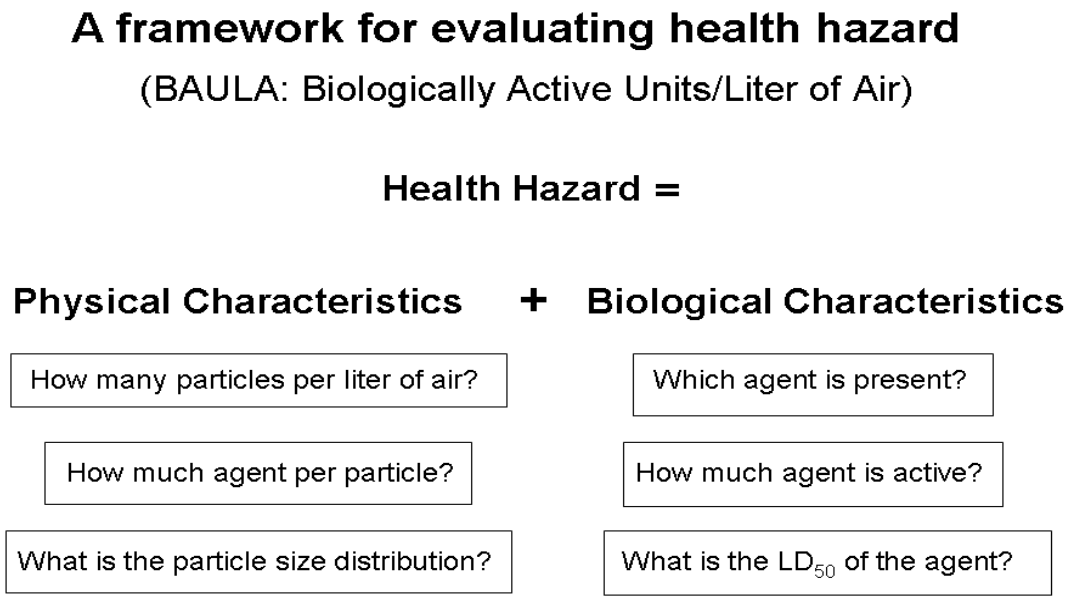


FIGURE S.1 A framework for evaluating the health hazard posed by aerosolized biological warfare agent exposure.

Proposal of BAULA (Biologically Active Units per Liter of Air) as a new framework for evaluation of health hazard

The proposed unit of hazard—Biologically Active Units per Liter of Air ($BAULA_{Dae}$)—

is a particle aerodynamic diameter (D_{ae}) dependent measure of biologically active units per liter of air, such that a biologically active unit is the amount of agent required to have a certain probability of causing a negative health outcome. $BAULA_{Dae}$ incorporates the key information needed to estimate health hazard. Because different threat agents pose different hazards, biologically active units would be measured and calculated differently for each threat agent. For example, two agents present in the same quantity—as determined by CFU—but with differences in virulence would result in different values of $BAULA_{Dae}$ although their concentration as reported in the current unit, ACPLA, would be the same. Normalizing agent concentration to health hazard enables direct comparisons between all types of agents.

It is important to note that all of the factors in $BAULA_{Dae}$ cannot currently be accurately quantified for every biological agent. For example, the dose required to sicken 50 percent of a particular exposed population is not known for every agent. Nor is it known, for every agent, how health hazard varies as a function of location in the respiratory tract. Extrapolation of animal data to human effects has uncertainties. In addition, obtaining non-human primate data of adequate precision and accuracy is extremely challenging but required due to the low natural occurrence of many of the pathogens of military concern. While the information used to calculate the health hazard may not be ideal (for example, LD_{50} levels determined in animal studies), even reasonable estimates of health hazard would improve the utility of the information given by detectors compared to a unit that does not even account for activity, much less virulence. The BAULA framework illustrates what type of information would be needed if detectors were able to provide warning not only of agent presence, but also of biological activity that can be related to health hazard. If it can be agreed that this is the ultimate goal for BWA detectors, then the BAULA framework provides an appropriate standard for reaching this goal. Initially, referee systems, and subsequently detector systems, can be developed with the new unit as their reference point, which will guide research and development and assist in identification and prioritization of knowledge and data gaps. Until these gaps have been filled—and some of these data (e.g., non-human primate LD_{50}) may never be obtained—it will be necessary to make appropriate assumptions to effectively use the unit and the framework.

Application of the BAULA Framework to Detector Assessment

The effective evaluation of detectors requires an effective set of test protocols and requirements.[2] During a test, the referee system, which does not have to evaluate the aerosol in real time and may consist of several different instruments, is used to provide an accurate measure of what the detector is being exposed to during testing. Because $BAULA_{Dae}$, like ACPLA, requires assessment of the physical properties of the aerosol, existing referee equipment can be used to quantitate the physical characteristics of the BWA aerosol. BAULA also requires biological activity to be assessed as a surrogate indicator of infectivity. The information acquired from these measures will then need to be related back to a chart providing population level lethal dose rates to determine $BAULA_{Dae}$.

Detector developers and testers operate at the interface of the physical and life sciences; both physical measures (e.g. particle size, presence of an agent) and biological measures (e.g.,

[2] For the purposes of this report, the term "detector" is used to describe equipment that is under evaluation or used in the field, "referee" corresponds to the description provided here, and "instrument" is used generically to describe the equipment and components in either detector or referee systems.

viability, virulence, host susceptibility) are needed to fully characterize the hazards of bioaerosols. Currently, and for the foreseeable future, there are insufficient scientific data to have an absolute performance measure for all detectors against all biological agents. Despite this limitation, there is sufficient scientific evidence to allow effective testing, particularly through the comparison of relative performance. These comparisons can be used to link to policy and requirements as well as to prioritize investments for improving detector performance. As an example, consider two candidate detectors and their performances detecting the bacteria *Bacillus anthracis* and *Francisella tularensis*. (Dennis et al. 2001; Inglesby et al. 2002) If both agents are equally likely to be used by an adversary, and data suggest that Francisella causes infection at a lower dose, then the detector that is more sensitive to Francisella would be preferred. Over time, this framework could help direct detector research and development to focus on developing measurement techniques to monitor the specific physical and biological characteristics that have the greatest impact on the hazard posed by BWA.

It should be noted that real-time detectors themselves will not likely directly measure $BAULA_{Dae}$. Because detectors can be designed to measure fundamentally different qualities of the BWA (e.g., CFUs, PFUs, quantity of nucleic acid, immunofluorescence), their outputs are not directly convertible to $BAULA_{Dae}$, but can be inserted into the BAULA framework. Referee systems will quantitate the $BAULA_{Dae}$ level to which the detector is exposed. Reducing the challenge amount until the detector can no longer discriminate challenge from background noise would define the detector's minimum detectable concentration.

A major consideration in adapting current testing procedures to the proposed new framework is that it requires differentiation of active and inactive agents. Current detectors and referee systems measure either active agent (in the case of instruments that measure CFU, for example) or all agent (for example, instruments that measure immunofluorescence) but do not distinguish active from inactive agent. This limitation is currently circumvented by making the conservative assumption that all agent detected is active. However, as technology progresses and instruments are developed that can distinguish between active and inactive agent, it will be important to take into account both whether the detector and referee systems are measuring the same thing and how much of the sample is remaining active throughout the testing process. Loss of activity during the testing process could cause discrepancies between detector results and referee instrument measurements. For example, if the sample preparation and aerosolization processes result in a 50 percent loss in activity, detector A (that senses both active and inactive agent) may appear to have twice the sensitivity of detector B (that detects only active agent) if the referee system is based only on active agent detection. This may translate to an inaccurate assessment that detector A is more capable of detecting the amount of agent needed to pose a health hazard than it actually is.

Current testing protocols often require that the aerosolization process produce an aerosol with fairly narrow particle size distributions. Accurate assessment of health hazard would require the generation of particle sizes that reflect different health hazards; the eventual goal would be to develop detectors capable of characterizing these physical characteristics of the aerosol. For now, improvements in the referee system are needed to obtain the particle size data identified in the equation. In the long term, use of the BAULA framework may facilitate the development and deployment of detectors that can determine the sample's particle size distribution.

There is significant value in implementing the use of $BAULA_{Dae}$ in the near term, even though the scientific basis for quantitatively linking all biological agents to health hazard is currently incomplete. With appropriate documentation of standard operation procedures,

methods, and reagents, many of the measurements needed to assess BWA exposure in $BAULA_{Dae}$ units can be accomplished with current detection technologies. For instance, particle sizing and counting; collection rate; sampling time; concentration; and CFU, PFU, and toxin mass can all be implemented today. Adoption of $BAULA_{Dae}$ as the standard approach to determining hazard will highlight the necessity to develop accurate factors for converting CFU (or other direct measurements) to biologically active units, thereby providing a focus for where research would be most productive. Overall, although the proposed $BAULA_{Dae}$ unit is more complex than the current ACPLA unit, $BAULA_{Dae}$ has the advantage both of providing a valid basis for comparison across all biological agents and, when incorporated into the broader risk framework, of providing an assessment with true operational value: the risk to health of those exposed to biowarfare agents.

RECOMMENDATIONS

Recommendation A: **A unit of hazard should be adopted as part of the evaluative framework. The committee recommends the unit be a particle size (D_{ae}) dependent measure of biologically active units per liter of air ($BAULA_{Dae}$).**

Recommendation B: **In support of an overall DOD detector evaluation philosophy that relates health risks and aerosol exposure, the committee recommends using the relationship in Figure S.1 to provide a framework for relating health hazard and aerosol exposure and to identify the information provided by different types of detectors.**

Recommendation C: **Standardize testing procedures for evaluating detectors.**

C.1 **Aerosol challenges need to be well characterized, including D_{ae}, $BAULA_{Dae}$, $BAPLA_{Dae}$ (size-resolved number of Biologically Active Particles per Liter of Air), and material rendered inactive during the process of aerosol dissemination and transport.**

C.2 **Challenge biodetectors with aerosols of defined size distributions.** At least three challenge aerosols with different median aerodynamic diameters ***(D_{ae})*** should be used in chamber or component testing of detectors. These should be chosen to represent deposition in the three regions of the respiratory tract.

C.3: **The standardized unit of hazard, $BAULA_{Dae}$, and the broader evaluative framework for health hazard should be adopted as DOD-wide standards, including use in T&E and procurement. The method for implementing the unit and framework should be documented, externally peer reviewed, and published.** Revisions and updates should follow similar vetting processes so that calibration, referee instruments, and testing reagents are standardized and variation is identified.

Recommendation D: **Survey the literature to better understand the transport and inhalation of particles to improve selection of appropriate D_{ae} (median aerodynamic diameter) challenge aerosols for test and evaluation. Balance this knowledge with intelligence of possible threats.** Specifically examine whether to include testing of particles smaller than 1μm and larger than 10μm.

Recommendation E: **Maintain ability to learn from unanticipated events by archiving data.**

E.1. **Archive parameters, methods, measurements, and test conditions during T&E of detectors.**

E.2. **Consider archiving raw data collected by deployed systems to guide future development of detectors through performance evaluation.**

Recommendation F1: **For priority DOD scenarios, an evaluation of uncertainty in the science, measurements, and situational awareness should be conducted so that resources can be invested in reducing the largest uncertainties that impact decision making.**

Recommendation F2: **An applied science program should be designed and executed to obtain information that will both improve the accuracy of the equation and help evaluate threats and their inherent uncertainties.** This will require information about different biothreat agents, instrumentation types, and scenarios important to DOD.

1

Introduction

Biological warfare agents (BWAs) possess properties that complicate defensive measures against them. First, the effective concentrations can be sufficiently low that an airborne attack need not be obvious or even directly sensible. Secondly, latencies or incubation periods between exposure and the onset of physiological reactions or symptoms are typically long (hours to days). By the time symptoms appear and clinical diagnosis is possible, the most effective treatment period may have passed. Simple awareness of an attack allows for the possibility of taking preventive action, such as donning protective equipment, to minimize exposure and initiate timely treatment. Awareness of the type and extent of an attack would also permit commanders to assess impact of the attack on meeting mission goals. These considerations make the development of fast, reliable BWA detectors that can alert personnel to possible BWA aerosol exposures a priority for the Department of Defense (DOD).

The development of detectors capable of providing the protections described above is a complex process. This report specifically addresses one step in that process, that of performance validation (test and evaluation) of candidate biological aerosol detection systems. Candidate detectors are subjected to biological aerosol test samples and their responses are evaluated to identify those that meet currently established requirements, such as minimum detectable concentration thresholds. The committee was asked to evaluate agent-containing particles per liter of air (ACPLA), the current standard unit of measure for biological aerosols.

1.1 REQUEST TO COMMITTEE

The DOD Joint Chemical and Biological Defense (CBD) Program's current requirements for evaluating biological aerosol detectors are stated in Agent-Containing Particles per Liter of Air (ACPLA). ACPLA is not a measurement that can be made directly. It is inferred both from the measurement made by the referee instruments and by what is known about the test sample. For example, instruments may measure colony forming units (CFU) of bacteria, plaque forming units (PFU) of viruses, and nanograms or picograms of toxins in a given volume of air. The test protocol for evaluating detectors is often designed with a controlled release of a test agent or simulant that challenges the candidate detector. Referee systems are used to calibrate the source as well as the detection system being tested.[1] However, these systems are not standardized across all tests and evaluations done for DOD and are often appropriately designed to be as simple as

[1] For the purposes of this report, the term "detector" is used to describe equipment that is under evaluation or used in the field, "referee" corresponds to the description provided here, and "instrument" is used generically to describe the equipment and components in either detector or referee systems.

possible. Referee equipment may also include instruments to measure overall aerosol particle count, sample fluorescence, and other characteristics of the aerosol. Variation in both the methods of characterization and the units used to quantify the amount of airborne agent material has created a barrier to comparison and cross-correlation of quantitative test results.

To address these issues, the DOD Joint CBD Program seeks a standard unit of measure that can be used for biological material independent of the state of the material (aerosol or aerosol resuspended or captured in liquid) and independent of agent type (i.e., bacteria, viruses, or toxins). The department asked the National Academies to conduct this study addressing the use of measurement in the testing of aerosol detector, whether ACPLA is the most appropriate measure and what alternatives exist. If one unit of measurement is not obtainable, the DOD seeks to establish a relationship for comparison of different units.[2]

1.2 DETERMINING A STANDARD UNIT OF MEASURE

The charge was to determine whether it is possible to develop a single unit of measure for airborne biological agents that may include bacterial vegetative cells, bacterial spores, virions, and biological toxins. The ultimate purpose of this unit of measure is to aid in the evaluation of sensors that are designed to warn and provide a measure of protection against the risk of exposure. Modes of action and the nature of the health threat vary widely among the different possible agents, and may even vary with the mode of exposure to a particular agent. However, the different biological agents share a common feature, and that is the *hazard* they pose to exposed humans. This feature is also shared by pathogens associated with naturally-occurring epidemics such as SARS, avian influenza, or toxins associated with red tide. This report focuses on biological warfare agents, but the concepts presented are equally applicable to a broad range of airborne pathogens, biological toxins, and chemical agent aerosols.

The potential harm of a BWA attack depends on such factors as the type of agent, activity or viability of the agent, quantity released, and method and circumstances of dispersal. As an illustration, the received dosage of a biological agent, such as anthrax, from which 50 percent of the exposed population dies (lethal dosage 50 percent, or LD_{50}) can be obtained from multiple (e.g., internet) sources as a number between 5000-20,000 spores. What is not generally discussed in this type of reference is the dependence of the LD_{50} quantity on the specific strain of anthrax. For example, the Aum Shinrikyo cult conducted several unsuccessful biological warfare agent (BWA) attacks in Tokyo in the early 1990s. Only one of these attacks, in 1993, was even recognized at the time. The lack of harm and the failure to detect the attack were due in large part to the selection of a vaccine strain of *B. anthracis* as the agent. If the group had selected a virulent strain to distribute, the outcome could have been different. Thus, LD_{50} is a critical characteristic of a BWA, and one can quickly appreciate that in order to gauge the effectiveness of an attack, a detection system should not only measure the number of *B. anthracis* spores present but also provide a measure of both viability and virulence of the agent. If a unit of measure does not take biological activity of the agent into account, it will have little use as a measure of the hazard a released agent presents.

Despite the many sources of potential variability in BWA attacks, it is useful to consider an idealized detector to help define appropriate units of detector performance. If consensus can be reached on an appropriate method of quantification for BWA aerosol sensors, the new method

[2] See Appendix B for the full statement of task.

can serve as a reference point to guide research and development, and to prioritize knowledge and data gaps. The actual measurement and characterization of biological aerosols will always be constrained by current scientific understanding and technological capability. It is necessary to develop a unit that can be implemented with current capabilities, but that will be robust enough to accommodate improvements in instrumentation and changes in the understanding of BWA aerosols.

1.2.1 Biological Warfare Agents

This study seeks to address appropriate detector measures for traditional biological warfare agents (BWAs): bacteria (both vegetative cells and spore forms), viruses, and toxins. Each of these can be regarded as having an irreducible minimum unit:

- Bacteria—a single cell or spore;
- Viruses—a virion; and
- Toxins—a single molecule.

Although the three traditional classes of BWAs are our focus, some attention was given to making the overall framework relevant to broader classes of toxins, including chemically synthesized toxins, non-protein biological effectors, and pathogens that are not traditionally considered in BW including protozoa and fungi. The approach recommended in this report is sufficiently flexible to accommodate any future modification or expansion of the BW threat classes.

At present, the definition of chemical versus biological agents is based primarily on the method of production. However, this distinction may be blurred somewhat as the capability to synthesize biological toxins expands. Therefore, the categories of biological toxins and chemical warfare agents can seem similar, from the perspective that exposure effects of certain BW toxins, such as botulinum, may be similar to some CW nerve agents, such as sarin. However, another important physical distinction exists: while CWAs include both molecular vapors (gasses) and particles, *all* BWAs are particulate materials (powders, aerosols). None of the BWAs, including biological toxin compounds, has a measurable vapor pressure at normal temperature and pressure, and therefore BWA exposure by an airborne attack necessarily involves the release or dispersion of suspended particles as aerosols. Consequently, a physical description of a BWA release would include some enumeration of the quantity of particles that contain these irreducible agent entities per unit volume of air. (The different types or classes of BWAs are described in more detail in Chapter 3.) Currently, Agent-containing Particles per Liter of Air (ACPLA) is the DOD standard for describing the concentration of BWA aerosols for all agent types.

1.2.2 Characteristics of an Ideal Unit of Measure

What would an ideal detection system provide? To manage risk to troops or exposed populations, decision makers need a measure of the existing and future risk from an attack. During the attack, decision makers would need to know the identity of the hazardous biological

material and the concentration per unit volume of air. Later, it will be necessary to consider such additional risks as the propagation of the bioaerosol cloud, continuing exposure risks, and resuspension of settled material.[3] Determining the actual exposure to a BWA that troops are likely to experience from an aerosol attack is difficult to assess for several reasons, many of which will be discussed further in subsequent sections of this report. Specific agent type or strains; method of dissemination; method of preparation, meteorological conditions, viability, and infectivity as a function of particle size all affect the exposure experienced by troops. Comprehensive assessment of the threat is substantially complex, with significantly greater information being required of the detection system than only the quantification and identification of a particular class of agent.

The goal of this report is to address one small part of this picture by exploring the possibility of a standard unit of measure for accurate performance comparison of bioaerosol detection systems. The report argues that an ideal unit of measure would also set the foundation for using detection systems to measure health risk and facilitate informed judgments. The goal of this section is to illustrate why an ideal assessment of a BWA threat is inherently complex, and not all of the information needed to assess the risks is currently available. Any proposed unit of measure should not only be useful given current technological capabilities but also be capable of approaching this ideal goal as more information becomes available. This is not to suggest that definitive empirical information, for example, from non-human primate studies will be readily forthcoming due to the inherent challenges in such studies. In addition, limited opportunity exists for gathering information for agents of interest during naturally occurring disease outbreaks. Nevertheless, even imprecise measures of lethality would be helpful in setting priorities for how sensitive detectors need to be to different bioagents.

At present, exposures to bacteria, virions, and toxins are expressed in units that reflect the numbers of agent-containing particles per unit volume (typically a liter) of air. For bacterial agents, this quantity has been called ACPLA. Informally, the term has been extended to virus or toxin exposures as well, but the information contained in the unit does not adequately reflect the biological effects of the different agents. ACPLA says nothing about the number of agents to which a person may be exposed (because it does not account for how much agent is contained in each particle), nor does it provide insights into the nature of the exposure (i.e., the difference between large particles that will deposit agent in the nose or throat when inhaled, and smaller particles that may convey agent into the lower airways). ACPLA also fails to account for the dramatic differences in the health hazard posed by different agents; the recipient of an ACPLA count must know what agent is detected and understand the health hazard posed by that agent in order to determine the proper course of action (e.g. antibiotics will not be effective treatment for viral or toxin agents). ACPLA is, therefore, not suitable for use as a single unit of measure for reporting bioaerosol data.

Another possible unit, agent concentration in irreducible units (e.g., spores), suffers from similar issues as ACPLA. For example, assume a concentration of one anthrax spore per cm^3, which is equivalent to 1000/liter, or $10^6/m^3$, and that a single spore has a volume of 1 μm^3 (approximately true, on average). This means that an equivalent mass concentration would be achieved by one 10 μm particle per liter, or one 100 μm particle per m^3. The larger agglomerate particle will settle out of the air faster and will likely not be as persistent, nor be transported as far as smaller particles, although turbulence and convection will allow particles to be transported

[3] For more information about factors influencing a health risk assessment in a military context see *Strategies to Protect the Health of Deployed U.S. Forces: Analytical Framework for Assessing Risks* (NRC 2000).

much farther than simple sedimentation models may suggest. While they are airborne, the larger particles are less likely to be inhaled. As the particle size increases the point of deposition moves from deep in the lungs to the bronchi to higher locations in the nasopharyngeal system, significantly changing both the infectivity and the lethality of the attack. At the point where the aerodynamic drag force on the particle for normal breathing intake velocities is small compared to its inertia, the particle has a low probability of being inhaled at all.

An alternate representation of concentration of biological material in an aerosol is the number of colony-forming units (CFU) of bacteria or plaque-forming units (PFU) of virus or molecules or mass of toxins per unit volume of air. These measures better reflect the concentration of agent to which a person may be exposed and provide the ability to determine whether an agent is capable of producing an adverse biological outcome, but still lack the detail needed to fully assess risk. As will be discussed in greater detail in later chapters, important factors, such as particle size, are lost with these units.

None of the above units of measure adequately achieves the goal of providing information about the potential health risk to exposed populations or troops, and an understanding of the possible health outcome could significantly affect a decision maker's course of action.

1.3 THE HEALTH PROTECTION OBJECTIVE

Exposure to biological agents can decrease personnel efficiency due to impaired health and the need to don restrictive protective equipment, whether individual or collective. Loss in efficiency may be suffered in the fighting force or in the support logistics and either can compromise mission objectives. A decision maker must weigh the perceived risks from exposure to biological agents versus the impacts of the intervention.

An adversary can launch a hazardous BW attack in many ways. From the defensive point of view, the end goal is always the same: to reduce health risk while maintaining the troops' ability to carry out their missions. Biological warfare agent (BWA) aerosol detectors play an integral role in the systems designed to defend troops. The role of biodetectors is at the community level (i.e., more of a public health tool than a predictor of individual disease) and should be viewed as supporting decision makers with a statistical estimation of health hazard. The probability of disease depends both on exposure and on host response, but the two factors are related because the nature of the exposure influences the host response. For example, the quantity of agent and the size distribution of the particles in which the agent is distributed are both critical physical characteristics influencing host response. Biodetection systems can help assess the probability of exposure and support approaches to reduce exposure. Knowledge of the population (e.g., vaccination history), in potentially exposed military personnel, can help estimate potential host response.

Ideally, bioaerosol detection systems provide information that explicitly links environmental measurements to potential health hazards in time, space, and disease potential for a specific population. As is argued in the coming chapters, there is only one relevant shared element in the various BWAs: all have a measurable effect on human health. This direct link to health risk is the basis of the final, recommended measurement framework that:

- establishes an absolute measure of performance;

- allows comparisons across a variety of detectors and agents;
- links health hazard to environmental measurement;
- can be implemented with current technology, although worst-case assumptions will have to be employed for some parameters that cannot currently be measured or estimated;
- supports and helps direct innovations in technology; and
- anticipates emerging scientific discoveries.

2

Exploring the Complexity of Health Risk

In developing a standard framework for evaluating the detection of biological aerosols, it is necessary to consider the biological and physical characteristics of the samples under study. Biological warfare agents (BWAs) are currently grouped into three categories: bacteria, viruses, and toxins.

2.1 BIOLOGICAL WARFARE AGENTS

2.1.1 Bacteria

Bacteria are single-celled microorganisms that are capable of replication independent of other living cells. Some can be grown in a simple liquid medium containing amino acids, salts, and other basic substances or on the surface of agar medium in a petri dish. The ease with which large quantities of pathogenic bacteria can be prepared in the laboratory contributes to their threat as biological weapons. Bacteria are often characterized by morphology (e.g., rod, sphere, or spiral), motility, and nutritional requirements (e.g., oxygen consuming). Bacteria can be pathogenic, parasitic, symbiotic, or free living, though combinations of these broad categories also exist (e.g., pathogenic, parasitic bacteria). Examples of bacterial pathogens and their associated diseases are:

- *Bacillus anthracis*—anthrax
- *Yersinia pestis*—plague
- *Francisella tularensis*—Tularemia

There are several approaches to quantifying bacteria, including flow cytometry, fluorescent labeling, and culture (i.e., colony counting). The ability to form colonies is one commonly used indication of viability for bacteria and their spores.[1] While there are other indications of viability, colony counting is the accepted "gold standard"; the resultant unit of measure is the colony forming unit (CFU). A limitation of the CFU is that several bacteria in close proximity on the plate will form a single colony (i.e., one CFU does not necessarily equal

[1]For the purposes of this report, the ability of bacteria to form cultures on artificial media will be considered a measure of viability. The committee acknowledges that this is more a direct measure of culturability rather than viability, but finds the uncertainty introduced by this issue falls into the broader category of uncertainty in threat assessment. See Box 3.1 for more information regarding uncertainty in bioaerosol detection.

one viable bacterium). An additional limitation is that some bacteria are non-culturable on artificial laboratory media, which could result in an underestimation of the quantity of viable bacteria present.

CFUs are determined using a standard culturing protocol (i.e., growth media, time, temperature), dispersal of a fixed quantity (i.e., volume, weight, or sample collected from a fixed volume of air) of sample on the growth media, and counting the number of visible growth patterns of the same type. By diluting or disrupting the sample and then repeating the standard protocol, it is possible to release bacteria that are clumped together to determine the total number of viable agent entities in the sample.

2.1.2 Viruses

Viruses are minute infectious agents that lack an independent metabolism and are able to replicate only within a living host cell. An individual viral particle (a virion) consists of nucleic acid (either DNA or RNA) and a protein capsid shell that contains and protects the nucleic acid; the shell may be multilayered. The host range of viruses is extremely broad; virions may infect bacteria (i.e., bacteriophages), plants, animals, and humans. Individual virions range from approximately 20 to 200 nanometers in size. However, without special treatment virions are usually associated with larger particles. Since viruses depend on a viable host cell for replication, naturally derived virions are often found in a mixed matrix with material in which they were grown. Viruses can be cultivated under controlled conditions in animals, eggs, and cell cultures. The material associated with virus-containing aerosol particles depends on how the virus was grown and processed before aerosolization. Therefore, aerosol particles containing viruses can be very diverse in size, chemical make-up and number of virions contained in an aerosol particle. Further complexity arises from the variation in potential host responses to viruses presented in such diverse size distributions and chemical matrices. Examples of viral pathogens and their associated diseases are:

- *Variola major*—smallpox
- *SARS associated corona virus*—severe acute respiratory syndrome (SARS)
- *Ebola virus*—ebola hemorrhagic fever

Aerosol particles containing viruses can be characterized using physical and chemical means as well as biological activity. Physical and chemical characterization methods are used to identify the presence of virus component materials. Viral nucleic acid is routinely analyzed by polymerase chain reaction (PCR). Capsid surface features are routinely identified by molecular recognition assays involving antibodies and other recognition moieties. These techniques do not assess whether the viruses are biologically active. (The ability of a virus to infect and replicate in a cell can be compromised by many environmental factors, including heat, sunlight, and humidity; different viruses are vulnerable to different environmental conditions.) Knowing whether the aerosol particle can establish an infection in a host cell culture or animal model is critical to determining the health hazard. Methods that are specific to a number of types of virus have been developed to characterize biological activity. These methods employ challenge of a known susceptible host cell culture, whole animal model, or other suitable host tissue, such as

embryonated eggs. The challenge is followed by incubation of the host or cell culture for a period of time after which biological effects are assessed. The biological effect is often an obvious end point, such as host death or cell death that produces a plaque (an area of dead cells) on a tissue culture monolayer. The results of tissue culture assays are presented in such units as plaque-forming units (PFU) per unit volume for tissue culture assays, while such units as 50 percent lethal dose (LD_{50}) or infective dose (ID_{50+}) per unit volume, or sero-conversion, are used for whole animal models. These assays involve surrogate host cells and animal models that are meant to simulate or predict infectivity, morbidity, and mortality in humans.

The above brief description of viruses, methods by which they can be characterized, and the challenges in predicting their impact on human health illustrates the difficulties associated with developing a unit of measure for virus-containing aerosol particles.

- A very large number of virions can be contained in a single respirable aerosol particle and the minute size of the viruses make optical counting or physical dissociation of viruses impractical;
- The virions may or may not be infective for a human host; direct assessment of human infectivity is currently virtually impossible to accomplish; and
- While a quantitative analysis of viral constituents (e.g., nucleic acids, surface epitopes) can be readily accomplished, relating analyte mass to infectivity is extremely difficult, even for well-characterized viral strains.

2.1.3 Toxins

Biotoxins, the third class of BWAs, are biologically derived substances that have a deleterious effect on an organism. Biotoxins range in size and composition from small molecular weight nonprotein toxins (e.g., aflatoxins) to large molecular weight protein toxins (e.g., botulinum). Biotoxins are naturally produced by a wide range of organisms, such as bacteria, fungi, algae, snails, and higher organisms. Advances in chemical synthesis and bioengineering have provided additional approaches to producing toxins that were originally isolated from naturally occurring organisms. The mode of production of a toxin and how it is processed affects the composition of the particle or other environment in which it is found. Unless a great effort is made to purify the toxin, it may be a minor fraction of the total mass of an aerosol particle. This presents a sensitivity challenge for direct detection of the toxin, but opens up additional opportunities for detection of toxin-related substances (e.g., castor bean DNA associated with ricin).

Toxins can be quantitatively characterized in terms of chemical composition and physical structure or biological activity. A number of analytical techniques, such as mass spectrometry, provide great selectivity and sensitivity in analyzing the chemical composition of toxins; their physical structure can be recognized by antibody-based assays. These techniques determine the mass of sampled toxin, from which the mass concentration (mass/volume) can be calculated. Once again, it is their biological activity that is of principal importance. In some cases, toxins can be deemed to be present by chemical analysis even after biological activity has been lost due to conformational changes or inhibition. Toxins perturb host processes in many different ways (e.g., diphtheria toxin inhibits protein synthesis whereas botulinum toxin blocks neuronal

transmissions); biological activity must be assessed by a method that mechanistically mirrors the toxicity pathway of the particular toxin in humans. Such in vivo and in vitro assays have been developed and the units of measure are often expressed in effective dose 50 percent (ED_{50}) per unit volume.

Toxin-containing aerosol particles present a relatively simpler case for characterization than that of bacteria or viruses. Nonetheless, complexity exists in host response depending deposition site in the respiratory tract, so aerosol particle size remains important. The host dose response curve and the effects of duration of exposure relative to total dose add additional uncertainty that must be addressed. At present it is not clear how the distribution of toxin molecules on aerosol particles will affect the health outcome.

2.1.4 Sampling and Analysis of Biological Warfare Agents

There are two somewhat disparate elements contributing to the challenge of bioaerosol assessment, which sensor development efforts attempt to combine: microbiological measurement and aerosol measurement. Starting with microbiological measurements, three approaches have been employed historically to indicate microbial organism presence or identity: (1) culturing, (2) nucleotide sequencing or sequence recognition, and (3) antibody-antigen reactivity (immunological response). In these broad categories many specific measurement techniques have been developed. For example, enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL) are two of the many possible examples of distinctive measurement protocols that share a common detection mechanism of antibody-epitope binding. Of these three basic categories, only culturing conveys an assessment of viability, or more generally, biological activity. Results from culturing can be difficult to quantify or automate and often require lengthy incubation times. The combination of these features makes this measurement approach impractical for use in autonomous, continuous-operation detection systems. It should be noted that, as a measure of viability, the ability to culture an organism is limited by the inherent challenges of growing viruses or bacteria in artificial environments. Because of its acceptance as a legal precedent for microbial identification, culturing will likely continue to be used for confirmatory analysis beyond the immediate detection system capability.

On the aerosol measurement side, three methods have been used historically to collect an aerosol sample from a volume of air: (1) filtration, (2) impaction, and (3) impingement. Filtration involves collection onto a filter substrate and is used to determine a time-averaged aerosol concentration. Filtration methods with adequate pore size will collect most particles larger than a minimum penetration size. Impaction uses the principle that an aerosol particle has significantly greater inertia than aerodynamic drag, making it unable to follow the air as it is deflected by an obstacle. This is exploited to separate the particle from the gas, typically onto a substrate. Impingement involves the transfer of a particle suspended in air into a liquid by impaction or by bubbling the air through the liquid. As with microbiological measurements, there are many specific embodiments of these three generic aerosol sample collection mechanisms and each has its unique characteristics. However, all methods require trade-offs that must be considered when developing and testing bioaerosol detectors.

A simple bioaerosol detection system will consist of one of the above microbiological measurement methods combined with an appropriate aerosol collection method. This basic approach is illustrated by the Department of Homeland Security (DHS) Biowatch program,

which couples aerosol filter collection with PCR, a technique that detects and amplifies specific DNA sequences. A strategy in which some aerosol property is monitored continuously is used for military applications. This monitoring does not necessarily require the collection of particles. Detection of a change in this property consistent with the presence of bioaerosols triggers the more intensive process of aerosol sample collection and microbiological measurement. One of the most successful aerosol monitoring methods to date has used optical spectroscopic properties of biological aerosols, especially ultraviolet excited fluorescence in conjunction with elastic scattering. Similar optical spectroscopic properties have also been exploited to provide a preliminary indication of the presence of bioaerosols at extended distances (i.e., provide some remote sensing or standoff detection capability).

It should be noted that current capabilities are constantly changing due to fairly intensive research and development efforts to introduce and validate alternative measurement techniques. On the microbiological side, proteomics has been pursued to create an alternative to antibody-based identification such as measurement of an array of specific epitope binding units other with aptamers and antimicrobial peptides. There are also several physical-chemistry-based approaches involving mass spectrometry, ion mobility spectrometry, and higher resolution optical spectroscopies, such as infrared absorption or Raman scattering. Some of these developmental measurement approaches can be used both for rapid detection of single particles and with a collected macroscopic sample. It is possible that one of these approaches, or combination of approaches, could exhibit sufficient discrimination to be used as the main microbiological detection mechanism, displacing the microbial measurement methods listed previously. For aerosol measurements, recent developments in air-to-air particle concentrators, particle charging and manipulation, and selective particle collection may also augment current BW detection system capabilities.

For the purposes of this report, it is important to remember that whichever collection method is used and whichever physical characteristics of the agent are measured, it is necessary to be able to relate the measurements made by a detector to the health hazard posed by the aerosolized agent.

2.2 PROPERTIES OF AEROSOLS

2.2.1 Transport and Dissemination

The likelihood of disease in a person exposed to a BWA aerosol is dependent in large part on the properties and characteristics of the aerosol itself. Biological agents, including biotoxins, behave as particles when they are disseminated in air. Airborne particle size depends on the formulation of the agents and the method of dissemination. Large particles sediment more quickly than smaller ones, reducing the time they are likely to remain airborne.(Seinfeld and Pandis 2006) Settling velocity, while important, does not entirely determine how long particles will remain airborne, or how far they will be transported as shown in Figure 2.1. It has been suggested that based on their high settling velocities, large particles containing BWAs do not pose a threat except very near the source. If this were true, pollen-induced allergic rhinitis (e.g., hay fever) would be less common, because pollen grains range from 20

μm to 100 μm in diameter, corresponding to settling velocities ranging from 1 to 30 cm/s.[2] However, atmospheric turbulence and convection enable long-range transport by raising particles to altitudes well above those at which they were released. While most large particles may land close to the source, some are transported much farther than the terminal settling velocity would suggest.

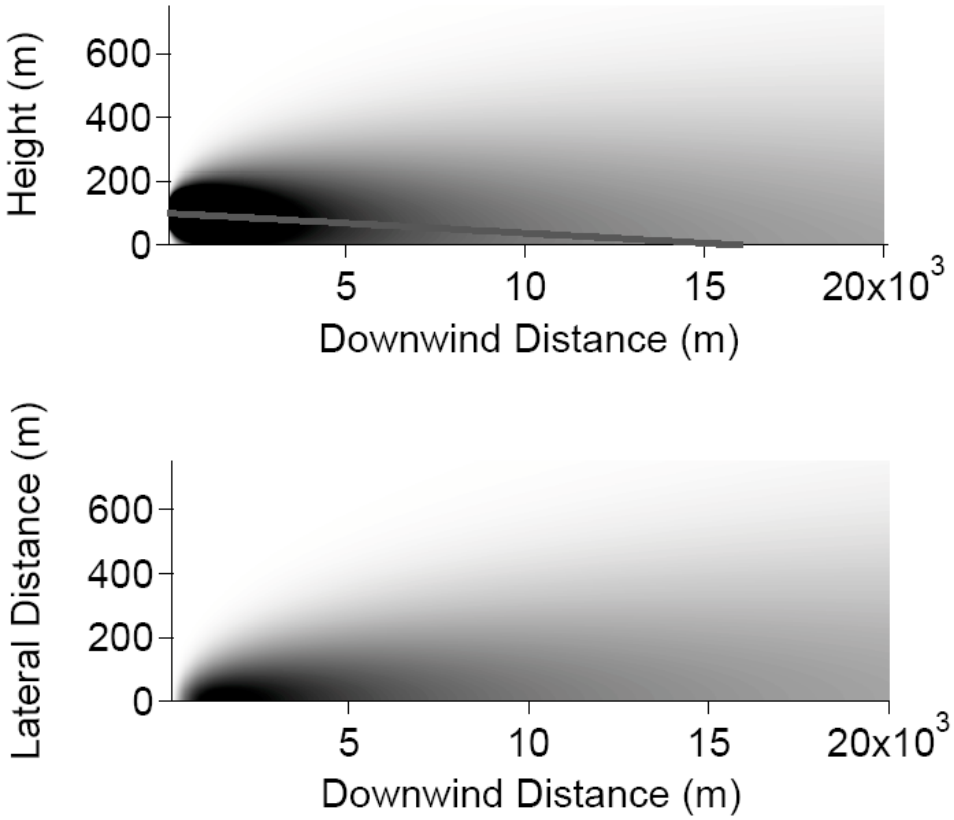


FIGURE 2.1 Particle sedimentation over distance. The top figure shows a cross-section through the center of the plume from a point release of 25 μm particles at a height of 100 m with a mean wind speed of 3 m/s and an eddy diffusivity of 10 m^2/s. Shading denotes relative particle concentration. The gray line shows the trajectory of the particles in the absence of atmospheric turbulence. The bottom figure shows the concentrations at ground level. Turbulent dispersion causes the ground level concentration to peak well upstream of the point predicted by the kinematic model, but also allows particles to reach distances well downstream of the point estimated by fall time alone, and to be dispersed well to the side of the original release trajectory.
SOURCE: Courtesy of Richard C. Flagan.

Aerodynamic diameter (D_{ae}) depends on several particle characteristics, including size, morphology, relative hygroscopicity, and density. The longer a particle remains airborne, the greater the potential for human respiration, so the aerodynamic diameter is directly related to risk of bioaerosol exposure. Diameter also greatly influences where a particle will be deposited in the

[2] Birch and other tree pollens have been detected in Finland weeks before the trees there begin to flower, but after flowering has occurred farther south in Europe. Long-range transport of the pollen grains is the likely cause for this early appearance.(Hjelmroos 1991), (Matikainen and Rantio-Lehtimäki 1998)

respiratory tract and thereby further links this physical characteristic with health risk. A number of aerosol collectors have been designed to collect fractions of the aerosol that crudely relate to their behavior in the respiratory tract; others (such as cascade impactors, aerodynamic particle sizers, optical particle counters, and aerosol mass spectrometers) segregate or measure particles in a number of size fractions from which more detailed assessments of respiratory tract deposition patterns can be determined.

2.2.2 Deposition in the Human Respiratory Tract

Particle size is a major factor determining the probability that an inhaled particle will be deposited in a specific region of the human respiratory tract. The other two major factors affecting the deposition pattern of inhaled particles are the airway morphology and breathing physiology.

The human respiratory tract can be divided into three anatomical regions as shown in Figure 2.2: (1) the extrathoracic (ET), or head airway, is the entry to the respiratory tract and the first defense against hazardous inhaled material; (2) the tracheobronchial (TB) tree, or conducting airway includes the trachea and 16 generations of branching airways. Gas exchange takes place in (3) the pulmonary region (P), which consists of alveolar ducts and alveolar sacs. Most people breathe through the nose during rest or light exercise, but switch to a combination of oral and nasal breathing during heavy exercise or work, because resistance through the oral airways is much lower than through the nasal airways. Some people are habitual oral breathers even at rest.

When an airborne particle is transported near a person, it may be inhaled and enter the respiratory tract through either nasal or oral passages. The ability of the particle to enter the head airway, labeled its inhalability, is a function of its aerodynamic diameter.(Phalen et al. 1986) Based on experimental data obtained largely in aerosol wind tunnels, the inhalability (which is a fraction of airborne particles entering the human airway) is near 100 percent for particles smaller than 5 μm. The inhalability decreases as the particle size increases and stays at 50 percent for particles greater than about 50 μm.

Once a particle enters the body through either the nose or mouth, it is deposited in different regions of the respiratory tract: larger particles deposit by inertial impaction or sedimentation, smaller particles deposit by diffusion. Electrostatic effects may enhance or modify deposition of charged particles of any size. Condensation of water in the humid environment of the respiratory tract may cause particles to increase in size, changing the way they move and deposit in the respiratory tract. The efficiency of particle deposition and the spatial distribution of deposition in the human respiratory tract have been measured experimentally in human volunteers using test aerosols, usually comprised of spherical particles tagged with radiolabel. Computational models have also been developed to estimate deposition in each airway region by each of the deposition mechanisms based on known properties of the aerosol particles. These models have been used to predict the aerosol deposition patterns in human lungs.

Lung deposition models, such as those developed by the International Commission on Radiation Protection (ICRP 1994) and National Council on Radiation Protection and Measurements, (NCRP 1997) are based on airway anatomy and breathing patterns of standard men. These models have been used extensively to estimate radiation dose from exposure to

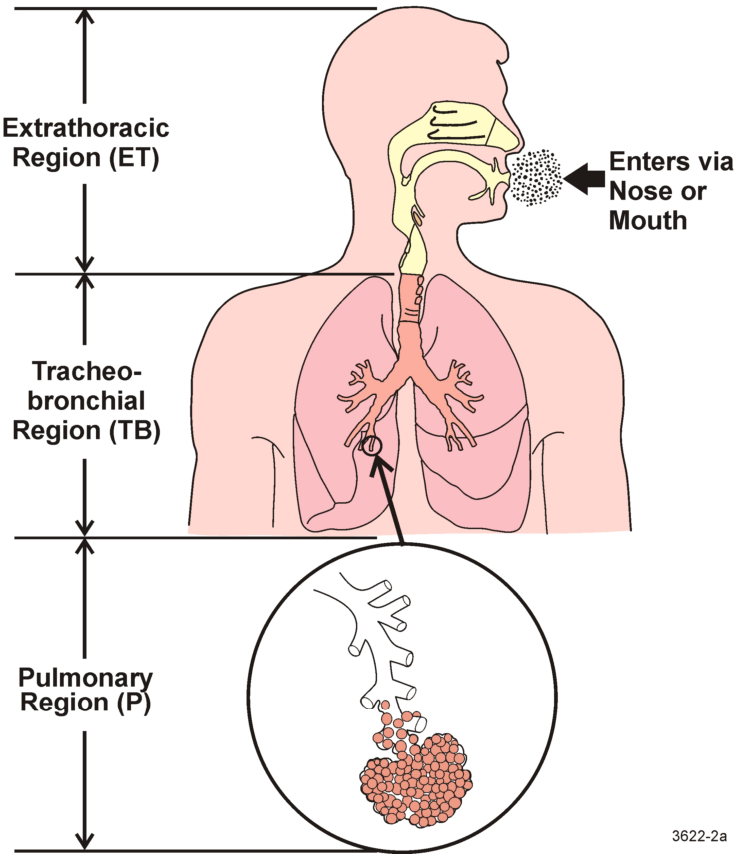


FIGURE 2.2 Schematics of the human respiratory tract.
SOURCE: Courtesy of Yung Sung Cheng.

radioactive aerosols. Figure 2.3 shows an example of the deposition fractions as a function of aerodynamic diameter in men with *nasal* breathing for a tidal volume of 1.25 L/breath and 20 breaths per minute. Figure 2.4 shows an example of the deposition fractions as a function of aerodynamic diameter in a man with *mouth* breathing for a tidal volume of 1.25 L/breath and 20 breaths per minute. With mouth breathing, deposition in the oral airway region is much lower than in the nasal passages for particles of all sizes between 0.01 and 10 than with nasal breathing. As a result, there is substantial increase in deposition in the TB and P regions when *mouth* breathing is the primary inhalation method. Therefore, aerosol delivery of pharmaceutical agents to treat lung diseases or to target systematic effects uses the oral delivery route. In the battlefield, oral breathing is likely during light to heavy workload, increasing the risk of lung deposition for agent-containing particles of all sizes.

2.3 BIOLOGICAL EFFECTS OF INHALED PARTICLES

Because particle size determines where aerosol material deposits in the respiratory tract, it is an important factor in predicting the health consequences of exposure to aerosolized bacteria, viruses, or toxins. However, there are only a handful of experimental studies in the medical literature that describe the effect of particle size on the nature of disease resulting from inhalation

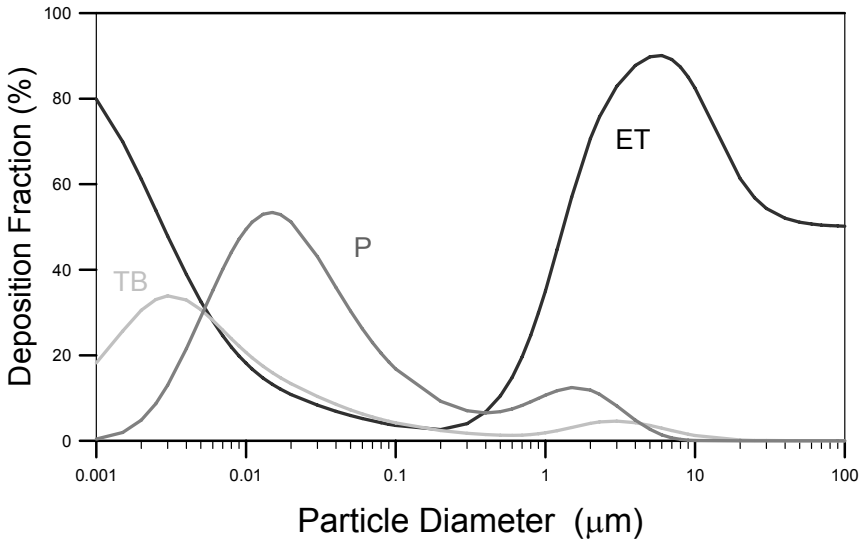


FIGURE 2.3 Distribution of particle deposition for different regions of the respiratory tract system for 100 percent **nasal** breathing. For bacteria, the relevant region is 1 μm and larger. For virus and toxins, dimensions much less than 1 micron are relevant. For particles larger than 0.02 μm in diameter, deposition in the extrathoracic (ET) region increases as particle size increases up to 8 μm. ET deposition also increases when particle size decreases from 0.02 μm. Increased deposition of the larger particles is driven by an inertial mechanism, and deposition of the smaller particles is a result of diffusion. ET deposition decreased slightly for particles greater than 6 μm, because of decreasing inhalability of large particles. Particles greater than 6 μm in diameter deposit primarily in the nasal airways with little penetration to the lung. Particles in the size range between 0.002 and 5 μm can penetrate into lower airways and deposit in the pulmonary regions. The data were calculated using the LUDEP software (NRPB, Oxon, UK) based on the ICRP model. (ICRP 1994)
NOTE: ET = Extrathoracic region, P = Pulmonary region, TB = Tracheobronchial region.
SOURCE: Courtesy of Yung Sung Cheng.

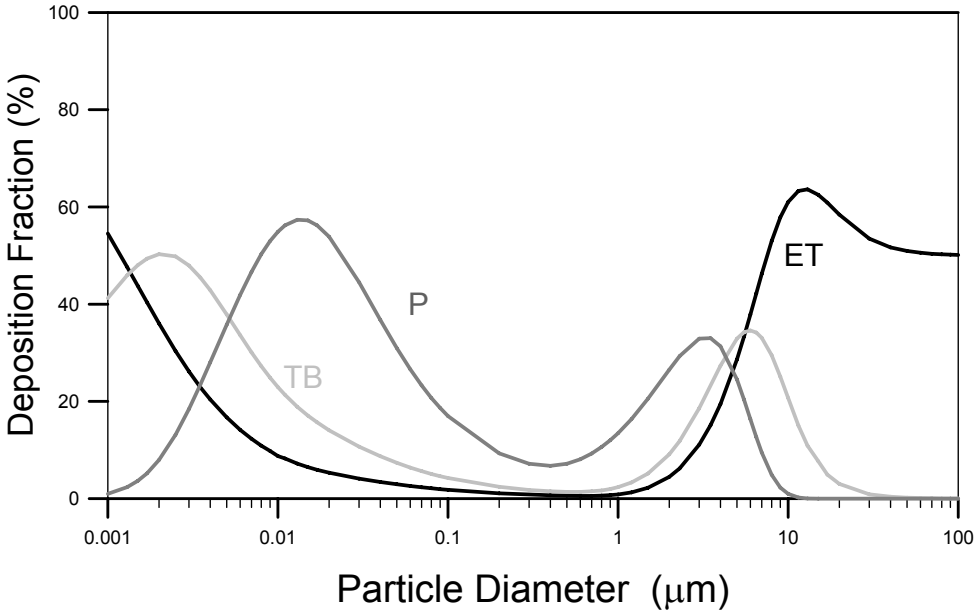


FIGURE 2.4 Distribution of particle deposition for different regions of the respiratory tract system for 100 percent **mouth** breathing. For bacteria, the relevant region is 1 μm and larger. For virus and toxins, dimensions much less than 1 micron are relevant. The data were calculated using the LUDEP software (NRPB, Oxon, UK) based on the ICRP model (1994).(ICRP 1994)
NOTE: ET = Extrathoracic region, P = Pulmonary region, TB = Tracheobronchial region.
SOURCE: Courtesy of Yung Sung Cheng.

of a biological aerosol and most were published before 1960. In the first report to examine this question from the point of view of BWAs, laboratory animals were exposed to radiolabeled spores of a simulant for *B. anthracis*, of various mass median aerodynamic diameter (MMAD).(Harper and Morton 1953) That study showed that a large fraction of particles in the 1-4 μm MMAD (in guinea pigs) or 1-6 μm (in monkeys) size range entered the lungs, while larger particles were almost entirely retained in the head and trachea. Even though deposition patterns in humans and various animal species differ because of variations in respiratory tract anatomy and breathing dynamics, experimental studies are in agreement that the infectivity of an aerosolized pathogen is greatest when it includes significant numbers of 1-5 μm particles that can reach the bronchioles and alveolar spaces.(Druett et al. 1953; Druett et al. 1956; Druett, Henderson, and Peacock 1956; Schlesinger 1985)

The lower probability of disease initiation per inhaled organism for particles of greater than 5 μm results from a combination of factors. First, the upper airway presents a much smaller target for deposition than the huge total surface area of the bronchioalveolar spaces. In addition, particles that deposit above the level of the respiratory bronchioles encounter a layer of mucus that limits their contact with epithelial lining cells. Those depositing above the respiratory bronchioles and below the trachea are also subject to the sweeping action of cilia that propel them upward out of the lung for removal by coughing or swallowing.

These larger particles cleared from the ET and P respiratory regions will mostly be ingested. This introduces another disease pathway that strongly overlaps alternative BW attack modes of food and water source contamination. For this reason, pathogenesis of the digestive

system was not specifically explored in this report, but may be particularly relevant to toxin agents. Although this section focuses on respiratory system response as a main pathway for airborne agents, the framework recommended here is easily extended to additional routes and mechanisms of disease. Similar considerations of efficacy as a function of particle size for cutaneous or ingestive routes likely exist. Prior literature or experimental data on these routes are even less prevalent than for respiratory effects.

By contrast, the greater pathogenicity of a microbe or toxin delivered in a 1-5 μm particle reflects its ability to reach the large exposed surface area of the alveoli that lacks both a mucus barrier and ciliated epithelium. Instead, such particles are cleared by local macrophages that may actually serve as sites of replication for such pathogens as *Bacillus anthracis*, *Francisella tularensis, Mycobacterium tuberculosis*, and a number of viral agents. In the case of inhalational anthrax, when the anthrax spores reach alveolar spaces, infected cells are cleared by the immune system into the mediastinal lymph notes, resulting in more severe disease than when the agent is confined to the upper respiratory tract and the agent is cleared to the cervical and peribronchial lymph nodes.(Druett et al. 1953) Even though pathogen-containing particles larger than 5 μm MMAD have a lower probability per particle of initiating infection than smaller particles, they are still capable of causing severe or fatal disease. The illness induced by large particles in laboratory animals differs in its clinical features from that produced by a small-particle aerosol. It is generally characterized by a lower mortality rate and usually, but not always, by a longer incubation period. For example, a 1-5 μm MMAD aerosol of *B. anthracis* caused hemorrhagic mediastinitis in rhesus macaques, similar to inhalational anthrax of humans, but delivery of the same agent in 12 μm particles resulted in massive edema of the soft tissues of the head and neck, presumably as a result of the initial spread of infection to cervical lymph nodes.(Druett et al. 1953) Similarly, while both small- and large-particle aerosols of *Yersinia pestis* caused fatal disease in guinea pigs, an aerosol composed of single organisms produced bronchopneumonia, leading to septicemia and death. A 12 μm MMAD aerosol actually produced septicemia more rapidly, but without causing pulmonary disease.(Druett, Henderson, and Peacock 1956) Experiments using aerosolized *Brucella suis* ranging in size from single organisms through 12 μm particles showed that the smallest particles were 600-fold more likely to initiate disease, presumably because they were able to replicate more readily after depositing on the vast exposed surface of the pulmonary alveolar epithelium.(Druett et al. 1956) Similarly, monkeys exposed to aerosols of *F. tularensis* in which mean particle sizes were 2.1 or 7.5 μm became ill and died more quickly than those exposed to the same agent in 12- or 24-μm particles.(Day and Berendt 1972) As might have been predicted, the first group developed a diffuse pneumonitis, while the second showed massive infection of the upper airway. Another study compared the effect of small- and large-particle aerosols of influenza virus in mice and found that a much lower delivered dose of the small-particle aerosol was required to initiate disease.(Hatch 1961)

Particle size is also a factor that determines the biological effects of an aerosolized toxin, especially if the substance exerts its damaging effects directly on the lining of the respiratory tract. A recent study using the toxin ricin showed that a small-particle aerosol caused lethal illness in guinea pigs, while the same dose of delivered material in the form of 12-μm particles did not cause death.(Roy et al. 2003) Although ricin-containing particles presumably cause mucosal injury wherever they are deposited in the respiratory tract, deposition of large particles in the upper airway was apparently less damaging to the animals' ability to breathe than the deposition of small particles in the linings of small bronchi and alveoli, which led to diffuse airway obstruction.

Once particles deposit on the surface of the respiratory tract, they do not necessarily remain intact; they may absorb water and disperse into their smaller component parts. Therefore, it follows that the threat to health posed by an aerosol of bacteria, virions, or toxins would be greater as the average number of infectious agents or the mass of toxin contained in each delivered particle increases. This logical conclusion was verified in the only experiment of its kind to be found in the medical literature; guinea pigs were exposed to aerosols of *B. anthracis* of different particle sizes, and the average number of spores per particle was controlled by adding dextrin to the suspension before aerosolization.(Druett et al. 1953) The investigators found that different combinations of particle size and the number of spores per particle could produce similar health effects, and concluded that " to achieve the same mortality, the total number of spores presented to the animal, in particles of a given size, must be the same, irrespective of the number of particles carrying them." This result suggests many different factors contribute to the *nature* of the disease produced by an aerosolized microbe or toxin, including the identity of the agent, the vulnerability of the host, and the size of the aerosol particles. The *severity* of the disease will increase with the average number or microbes or mass of toxin in each particle. However, when an aerosol contains a variety of particle sizes, those in the range of 1-5 μm will always pose the greatest threat, even if they represent only a minor fraction of the inhaled material.(Hatch 1961)

As demonstrated in the above section, a limited number of reports, mostly published before 1960, have shown that particle size plays an important role in determining the probable health outcome after exposure to BWA aerosols. More research in this area using modern laboratory methods is clearly needed to better understand that role. For the detector testing community, this has implications for test design. As more information becomes available, the committee recommends that test guidelines be updated, published, and peer reviewed to reflect the improved understanding of particle size and health effects.

2.4 PARTICLE SIZE AND AEROSOL COLLECTION

Aerosols are described physically in terms of the aerodynamic diameter distribution of particles. For biological aerosols, the diameter and viability may be greatly influenced by air temperature and relative humidity. The biological content per particle is also important, as both size and biological content are needed to link to health hazard and potential mitigation strategies.

Monitoring particle size distribution presents technical challenges for testing of bioaerosol detectors. Most current detector collection systems capture particles over a range of diameters, for example particles smaller than 10 μm diameter (PM10), which can penetrate beyond the head and thoracic regions during mouth breathing, and particles smaller than 2.5μm diameter (PM2.5), which are respirable aerosols that can penetrate into the lower airways during nasal breathing. These characterizations do not, unfortunately, provide sufficient information to assess where in the respiratory tract inhaled particles will actually deposit. Bioaerosol samplers based on inertial deposition (e.g., slit-to-agar samplers) collect only particles larger than a critical aerodynamic diameter, while excluding smaller particles that may contain spores, virions, or toxins. In the study of hazardous components of the environmental aerosols, smaller particles have been shown to have disproportionately large health impacts. Several studies have suggested that surface area (Oberdorster 2000) or number concentration (number/cm^3)(Donaldson et al. 2000) may better represent the health impacts of such particles than does the particle mass.

Once a collection system has concentrated the aerosol into a single sample, it is no longer

possible to reconstruct the size distribution. Hence, the potential distribution of deposited particles in the respiratory tract cannot be accurately estimated or used to inform response priorities. The collection system can be conceptualized mathematically as a transfer function that blurs or integrates over a range of particle sizes. Therefore, performance of a detection system depends on the distribution of particle sizes, and this system bias should be determined as part of test and evaluation. Figure 2.5 illustrates a model system with good performance to a uniformly distributed 1-5 μm aerosol but with unacceptable performance to a 1-10 μm aerosol.

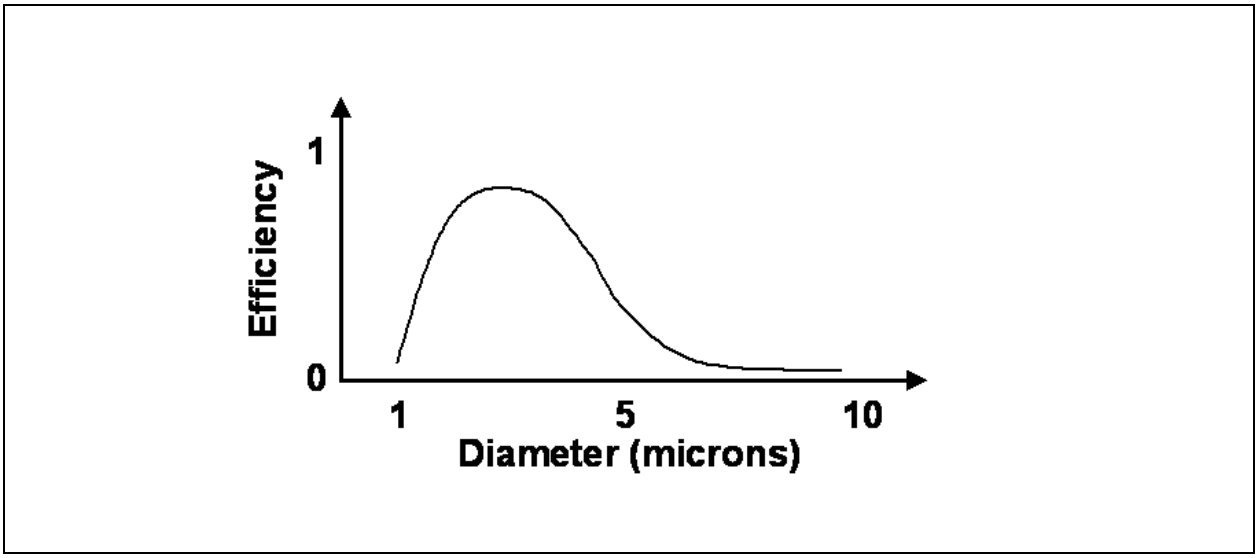


FIGURE 2.5 Illustration of a transfer function of a hypothetical detection system. After collection it is not possible to reconstruct the size distribution. In this example, particles with an aerodynamic diameter less than 1 or greater than 5 μm will not contribute to the signal.

2.5 CURRENT UNITS OF MEASURE AND HEALTH HAZARD

Conceptually, it is now possible to see more clearly the limitations of the current standard unit of measure for biological aerosols. The Agent Containing Particle per Liter of Air (ACPLA) unit has become the de facto standard for measuring performance of biodefense aerosol detection systems. Independent of specific implementation, ACPLA is the number of particles that contain at least one biological agent in a liter of air. ACPLA appropriately recognizes that particles with less than one biological agent (i.e., zero) are not a biodefense concern. However, there are limitations to using this unit of measure. Consider whether ACPLA accurately estimates exposure to biological warfare agents. ACPLA is in practice inferred from measurements of CFU, PFU or micrograms of toxin. Measures of CFU or PFU provide information about the number of particles containing culturable agents, but this may not yield the total number of agent units (exposure or dose) that is important for estimating health consequences. As an example, a measure of 10 ACPLA in reference to anthrax could equivalently describe either 10 individual spores per liter or 10 particles of size 7 μm per liter, each containing over 100 spores. In terms of irreducible units (spores), the concentration can vary by orders of magnitude for the same ACPLA designation.

The distribution of the biological content is also important. A thousand bacteria in a single 10 μm particle may represent a different health risk than a thousand 1 μm particles each with one bacterium. Obviously, if the bacteria are not viable or have lost the ability to be infective or pathogenic, the health hazard has changed dramatically. Current research is suggesting that nonviable content in the aerosols—including lysed cellular material—may contribute to the health risk or, to the contrary, provide benefit to the immune response in some cases. The currently used unit of measure, ACPLA, and current state-of-the-art detection capabilities, cannot take these factors into account.

A further limitation of ACPLA is that it does not contain any indication of particle size and particle size distribution, characteristics of the aerosol clearly important in evaluating health hazard to humans. For aerosolized particles with biological content, the aerodynamic diameter is a critical parameter in determining the final site of deposition in the respiratory tract and must be considered.

In light of these limitations, the committee developed a new, robust framework for evaluating the health hazard posed by biological aerosols. The next chapter describes the recommended framework and its mathematical and conceptual reasoning.

3

Building the Framework for Evaluating Health Hazard

Ultimately, the purpose of aerosol biodetector systems is to provide for early warning of an airborne health hazard. Biodetection should, therefore, focus on those aerosol particles that have the potential to cause deleterious biological effects in humans. Since biological activity is the principal focus, bioaerosol measurements should be reported in terms that quantify this characteristic. A measure based on anything but biological activity would focus attention away from the central purpose of biodetection—identifying and quantifying health hazard. Thus, biological activity provides a convenient starting point for developing a framework for evaluating risk given the wide range of biological agents and detection methods. The approach described here is general and applicable to research beyond the DoD T&E community.

The health impact of BWA exposure is affected by both the physical and biological characteristics of the aerosol. To be of value across the full spectrum of biological threats, an evaluation framework should include the principal physical and biological factors affecting health hazard. In the previous sections, it was concluded that the two main aerosol cloud characteristics affecting health hazard are (1) physical properties that determine site of deposition in the respiratory tract and (2) biological activity at the deposition site. A framework that incorporates information about these key determinants would allow an analytical assessment of health hazards. It would also provide an appropriate and interconvertible standard for the testing of biodetectors.

In the sections that follow, an approach for quantifying the health hazards from aerosols is presented. Existing measurement technologies that support the use of this framework are described. Finally, an approach to detector testing and the connection between health risk and detector testing is explored.

3.1 THE FRAMEWORK IN WORDS

Biological activity can be described as the concentration of biologically active aerosol particles (BAP) or total biologically active units (BAU) in a volume of aerosol cloud. The BAU can be made up of virions, bacteria, toxin molecules, or a mixture. Aerosol particles may also contain nonactive substances that affect assessment of biological activity, such as assay inhibitors, growth media, or salts. As discussed in the previous chapter, current research suggests that the number of BAU has greater influence on health risk than the number of BAP. However, this conclusion is based on minimal data for only one bacterial agent. The possibility exists that future research will identify threat agents for which BAP may strongly influence health risk (e.g., by determining the number of lesions formed). For this reason both primary and secondary units of bioaerosol activity measure are recommended below.

The uncertainty in relative importance of the number of aerosol particles deposited at a

site in the respiratory tract versus the total number of biologically-active units deposited is a minor aspect of the inherent uncertainty associated with characterizing the biological threat. A detailed discussion of the effect of biothreat uncertainty on biodetector testing is outside the scope of this study, however, a summary is provided in Box 3.1. Briefly stated, evaluating biodetector accuracy is severely constrained by inherent threat uncertainty (e.g., which strain will be encountered, what the particle distribution will be, what the agent will be suspended in). However, high precision in biodetector testing can be achieved by well-controlled system and component testing with simulants and well-chosen threat agent prototypes.

3.1.1 Normalization of Agent Concentration to Health Hazard

Biological activity with respect to the ability to cause an adverse health response is routinely characterized using controlled biological assays. The observed biological end point

BOX 3.1
Precision and Accuracy in Biodetector Testing

The number of different bacteria, viruses, and toxins that can cause adverse health effects is great. For each bacterial and viral type of agent (e.g., the genus and species *B. anthracis,* VEE virus), myriad strains with varying biological and physical properties occur naturally; anthropogenic effects, from agricultural use of antibiotics to genetic engineering, may alter natural strains. Some infective strains are culturable using artificial media; others will not grow in such an environment. Which specific microorganism will a biodetector actually be confronted with on the battlefield? The mode of aerosol dissemination (e.g., dry or wet dissemination, particle size distribution), suspension media (e.g., addition of fluidizers to prevent clumping, chemicals to absorb killing UV light and potentiate agent biological activity), and natural background materials (e.g., chemical pollutants, pollen, and ambient microbial flora) can greatly affect the nature and health effects of the aerosol cloud. It is not presently possible to fully test and characterize the accuracy of a detector against this vast array and combination of potential agents and aerosol presentations; nor could current detectors identify the entire range of agents, even if such testing were possible. Therefore, biodetector *accuracy* testing is inherently limited, in contrast to the great *precision* that can be achieved by employing well-controlled test procedures. A practical and useful level of accuracy can be approached by carefully selecting prototypical strains and bounding key aerosol parameters, such as particle size. A robust understanding of pathogenesis and phylogenetic diversity (e.g., selecting strains with conserved virulence epitopes) for a pathogen is critical to constructing such a practical level of accuracy in biodetection testing.

varies, depending on the mechanism(s) of pathogenicity for the particular agent and the assay system. Results of these assays are often expressed as a statistical function, such as the dose that is lethal, infective, or effective for 50 percent of an exposed population (LD_{50}, ID_{50}, or ED_{50}, respectively). Expressing biological activity as a statistical unit of health consequence provides the opportunity to "normalize" activity units across the spectrum of biothreat agents. One can compare the effects of a set number of LD_{50} units for several disease-causing microorganisms. For example, in vaccine efficacy testing, vaccine treatment groups are often challenged with 100 LD_{50} of agent. When articulating the performance of a biodetector, the threshold of detection for any number of agents can be expressed in terms of LD_{50} units. Converting the actual number of bacteria, viruses, or mass of toxin to units of biological activity provides for greater information

content and facile appreciation of relative hazard. In addition, normalized units of adverse biological activity enable comparison of health hazards across diverse modes of exposure (e.g., pulmonary, cutaneous, ingestion).

A statistical representation of dose response, such as LD_{50}, is of great use in describing health hazard, and some effective models and framework for dose response analysis have been developed. However, there are practical limitations in utilizing such statistical representations. Limitations include lack of knowledge of the shape of the dose response curve (e.g., probit slope) and fidelity of animal model data in representing human response. Lack of knowledge of the dose response curve can be a significant concern when low incidence effects are important. For example, a commander may be concerned about an LD_1 if the infectious agent is highly contagious, since a single infected person could pass the disease to others. For pathogens with large LD_{50} values (e.g., *B. anthracis*), LD_1 can vary greatly depending on the shape of the population dose response curve as well as specific host susceptibility (e.g., age, health, and immune status).

Biological activity assays are surrogates for human health effects. The assays employed for different biothreat agents vary in fidelity for predicting human disease. Therefore, the robustness of predicting health effects in humans for a particular agent, as well as for comparative assessments across agents, varies with the fundamental knowledge base for the agent as well as availability of suitable animal or *ex-vivo* models. The importance of understanding the mechanism(s) of pathogenicity for each biothreat agent, whether for estimating aerosol health risks, medical countermeasures development, or biodetector testing cannot be overstated. One of the benefits of adopting a detector evaluation framework based on biological activity is that it will focus research attention on areas where understanding of pathogenicity is poor. Use of *in-vivo* and *ex-vivo* data from multiple animal species and challenge modes (e.g., pulmonary, oral, cutaneous) can provide greater fidelity for estimating human health effects and hazards. There is currently a paucity of data for some pathogens for converting detector output to human health hazard. Knowledge gaps in human dose response and modes of action of pathogens, of which there are many, will be brought into clear view when BAULA is implemented. Currently, due to this lack of knowledge, field commanders "finesse" interpretation of biodetector outputs to guide operational decisions. Rapid progress is being made in characterization of pathogens of importance to DOD, due to investments by several federal departments, but generating detailed and accurate non-human dose response curves for all bioagents will be difficult and costly. Even relatively crude estimates of pathogenicity and infectivity, however, are arguably more useful than using a measurement system that implicitly assumes that all agents are equally hazardous.

3.2 PROPOSED FRAMEWORK FOR EVALUATING AEROSOLIZED BIOTHREAT AGENTS

The committee recommends that the following units be adopted as Department of Defense-wide standards:

$BAULA_{Dae}$. A primary unit of concentration is recommended for biological aerosols that quantifies Biologically Active Units per Liter of Air as a function of aerodynamic diameter ($BAULA_{Dae}$). This unit permits incorporation of a predictive model of the site of deposition sites in humans. Biological activity is itself a compound term that incorporates the quantity of active

agent, strain identity, and some probability of physiological response. The $BAULA_{Dae}$ provides key information needed to estimate health hazard. Biologically active units are measured and calculated differently for each threat agent, to provide a common measure of hazard for agents with disparate modes and levels of biological action, and to account for different activity levels for particles that deposit in different regions of the respiratory tract or otherwise affect human response. The biological activity is normalized to a 50 percent population activity end point. An example of the use of this unit in estimating the health hazard for an aerosol challenge with *Bacillus anthracis* spores would be as follows. An aerosol would be generated having a particle size distribution of 1-3 μm number median aerodynamic diameters; D_{ae} is 1-3μm. A volume of aerosol might be collected into a liquid and the original aerosol particles disrupted to yield individual spores. The spores could be quantified on agar plates yielding CFU/ml of collection fluid volume. The number of LD_{50} units contained in the original aerosol would be calculated based on air volume sampled, volume of liquid in which the spores were suspended, volume plated on agar plates, and estimated number of CFU in an LD_{50} for *B. anthracis.*

$BAPLA_{Dae}$. The committee proposes a secondary framework for bioaerosol measurement to quantify the size distribution of particles in an aerosol that contain biologically active material. This measure is the size-resolved number of biologically active particles per liter of air for an aerosol of a defined size distribution ($BAPLA_{Dae}$). This secondary unit of measure could be used to provide a comparative unit for historical comparisons with ACPLA measurements and in anticipation of the discovery that the number of sites where aerosol particles deposit biologically active units in the respiratory tract may need to be known to predict health hazard for some agents. As $BAULA_{\underline{Dae}}$ represents total agent units per liter rather than units per particle, the site information is lost when using $BAULA_{Dae}$.

$BAPLA_{Dae}$ would be determined in a manner similar to current methods for ACPLA, with the exception of employing a defined challenge aerosol particle size distribution and normalizing biological activity for each agent. In the case of a *B. anthracis* challenge, a defined volume of air would be impacted over time on a rotating slit impactor sampler. The number of CFU growing on the plate would be used to estimate the concentration of aerosol particles containing spores and adjusted for the number of CFU in an LD_{50} of *B. anthracis*. As with $BAULA_{Dae}$, measured or estimated values of LD_{50} for agents are needed to determine $BAPLA_{Dae}$ values. In addition, the combination of these two units, $BAULA_{Dae}$ and $BAPLA_{Dae}$, can also provide an estimate of the mean number of biologically active units per particle of size D_{ae} that contains any biological activity.

Over time, more will be learned about the relative health risks of BWA exposures, so that the quantity of an agent that represents one $BAULA_{Dae}$ may change for any given agent. Linking $BAULA_{Dae}$ to the probability of a negative health outcome, as will be discussed in the next section, allows for an adjustment of test requirements without fundamentally altering the unit.

3.3 A MATHEMATICAL DESCRIPTION OF BIOAEROSOL HEALTH RISK

The health *risk* presented by a bioaerosol includes two components: the *hazard* posed by the agent and the *physiological responses* of the individuals exposed to it. Thus, a cloud of smallpox virus is a *hazard*, but it may pose no health *risk* if all of the exposed individuals are effectively immunized against smallpox. What the committee is proposing in $BAULA_{Dae}$ is a unit that quantifies the *hazard* posed by a particular bioaerosol. The $BAULA_{Dae}$ hazard

framework (see Figure S.1, p. 7) can then be embedded in a full health risk framework that places the hazard measurement in the context of the likely physiological impact on the particular population in question. The final result provides an accurate assessment of health risk.

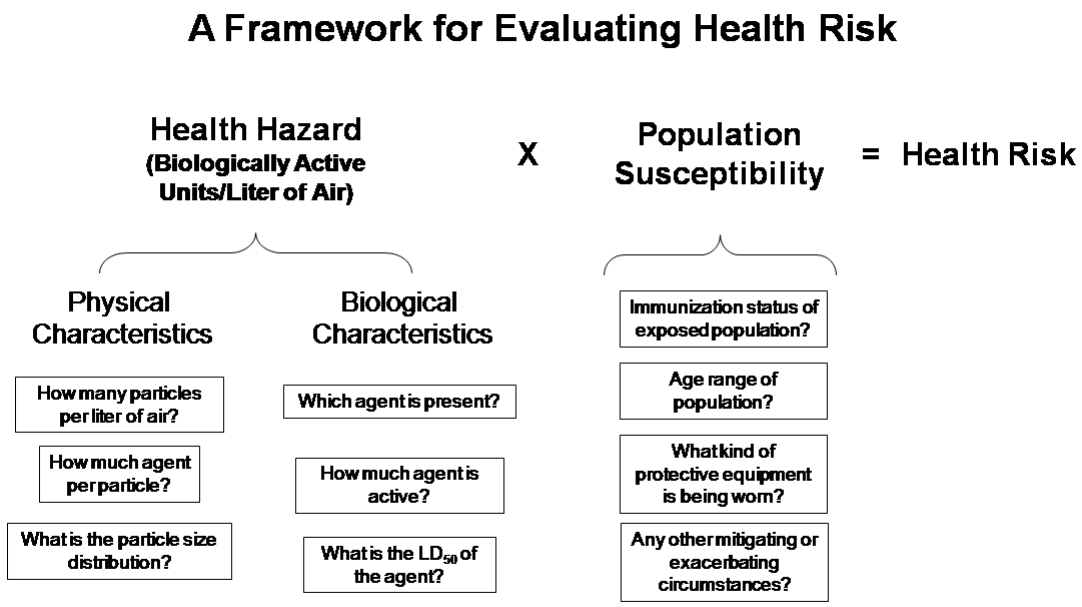


FIGURE 3.1 A framework for evaluating the health risk posed by aerosolized biological warfare agent exposure.

The committee performed a survey of common units of measure and concentration used in the bioaerosol community and found that these fell into four general categories. Table 3.1 summarizes the comparison of $BAULA_{Dae}$, $BAPLA_{Dae}$, and other common units. Note that the new units are the only ones that take into account the factors necessary to evaluate the actual health hazard posed by any BWA aerosol as described in Equation 3.1. In all other cases, the concentration measures do not provide enough information to adequately evaluate the health hazard.

The committee asserts that a framework that uses health hazard to compare biowarfare agents best addresses the charge presented by the statement of task to propose a unified method for comparing bioaerosol threats. However, as the equation describing the recommended framework shows, converting to a measure of health hazard is complex. Even a minimal estimate of health hazard requires accurate measures of lethality and infectivity, which have not been established for all possible biological agents. Furthermore, in a field environment, there is likely to be incomplete knowledge of how many other factors might affect health risk, from weather conditions, to host immune status, to precise strain characteristics. The committee is aware that even $BAULA_{Dae}$ and $BAPLA_{Dae}$ represent incomplete measures of health hazard, but propose that their application would focus attention on the most important factors affecting troop protection, factors that are not included in the current unit of measure.

TABLE 3.1 Comparison of Common Units of Measure for Biological Material

	Physical Characteristics		**Biological Characteristics**	
	Particle Size	**Total Amount of Active Agent**	**Activity of agent**	**LD_{50}**
Agent containing particles per liter of air	No	No	No	No
Particles/unit volume of air	No	No	No	No
Mass/unit volume of air	No	No	No	No
Viable organisms/unit volume of air	No	**Yes**	**Yes**	No
$BAPLA_{Dae}$	**Yes**	No	**Yes**	**Yes**
$BAULA_{Dae}$	**Yes**	**Yes**	**Yes**	**Yes**

The additional information provided by the units described above is apparent when viewed in the broader context of health risk evaluation. Figure 3.1 provides a framework for exploring the feasibility of developing a standard approach to measurement that directly addresses the primary concern, risk of adverse health outcomes. In the discussion that follows, we develop a mathematical framework for this representation of hazard. This mathematical framework will then be used to examine the performance of sensors in light of their ultimate purpose, which is to reduce risk.

Airborne biological agents exist as aerosols (i.e., as suspensions of particles: liquid, solid, or mixed-phase) in air. No aerosol, even the best calibration aerosol, is truly monodisperse (i.e., consists only of identical-size particles); all aerosols contain particles that are distributed over some range of sizes. To understand the dose response to aerosol exposure we must address the contributions of particles of different sizes by introducing a particle size distribution function. The number of particles per unit volume of air with aerodynamic diameters between D_{ae} and $D_{ae}+dD_{ae}$ at a point in space and time is

$$dN = n(D_{ae},x,y,z,t)dD_{ae} . \qquad (1)$$

Equation (1) describes the distribution of particles without reference to their agent content. $n(D_{ae},x,y,z,t)$ is the differential particle size distribution with respect to aerodynamic diameter of the particles (hereafter labeled the particle size distribution), and is determined by the formulation of agents, the nature of the release, and the atmospheric processes involved in transport and transformation of the agent cloud, as well as all other sources of particles. Definitions of terms used in this section may be found in Box 3.2. Even when an agent is present at dangerous levels, many or even most aerosol particles will be common, agent-free ambient particles, such as dust, pollen, fungal spores, other natural aerosols, emitted primary particles, and secondary aerosols formed from gas phase chemical reactions.

The units of n are {number of particles}/{unit volume of air}/{unit of aerodynamic diameter}(i.e., $m^{-3}\mu m^{-1}$). D_{ae} is the aerodynamic diameter of the particle, which corresponds to the diameter of a spherical particle with unit specific gravity (density of water) that would sediment at the same velocity as the particle. It is selected as the basis for defining the particle size distribution since, for all but the smallest isolated virions or toxin molecules, the aerodynamic diameter is the particle characteristic that determines the mechanism and efficiency with which ambient exposure translates into dose in the different regions of the respiratory tract or other parts of the body. dD_{ae} is a *differential* interval in aerodynamic diameter.

To determine the dose or instrument response, we will eventually integrate over particle size. We will, however, retain the differential form in order to maintain rigor as we consider the many factors that may influence the dose and human response. While present measurement methods may not be able to fully resolve this differential distribution, this mathematical formulation provides a base from which the approximations inherent in different measurement methods can be elucidated.

The amount of agent in the air at a point in space and time is best expressed as a concentration or quantity of agent per unit volume of air. Some of the sensors that will be examined in the discussion that follows may not directly measure the biological agent; instead, they estimate or infer exposure from the concentration of detected particles in the air. The human response to exposure to airborne agent depends on the particles that deposit into different regions of the body, especially the respiratory tract. As described in section 2.2.2, the International Commission on Radiological Protection (ICRP 1994) has generated a detailed human respiratory tract model that describes particle deposition into different regions of the respiratory tract (extrathoracic (ET), tracheobronchial (TB), and pulmonary (P) regions) as a function of particle size and breathing rate. That model forms a basis for estimating the probability that a particle of size D_{ae} will deposit into each of these regions, including effects of nasal or oral breathing as discussed in Chapter 2.

The number of particles per unit volume of air in the differential size interval that will deposit into any one of the regions of the respiratory tract if inhaled is given by the products of the probability of deposition in that region and the number concentration of particles in the size interval, that is.,

$$\begin{aligned}
&\text{Extrathoracic region:} && dN_{Pe} = P_e(D_{ae},Q)dN = P_e(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \\
&\text{Tracheobronchial region:} && dN_{Pt} = P_t(D_{ae},Q)dN = P_t(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \\
&\text{Pulmonary region:} && dN_{Pp} = P_p(D_{ae},Q)dN = P_p(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae}
\end{aligned} \qquad (2)$$

where $P_i(D_{ae},Q)$ denotes the probability that a particle with aerodynamic diameter D_{ae} will

BOX 3.2
Variables in the Health Risk Equation

D_{ae}	Aerodynamic diameter
t	Time
$Q(t)$	Volumetric inhalation rate
$\nu(D_{ae})$	Number of agent units of aerodynamic diameter D_{ae} [0, inf)
$P\,\nu(D_{ae})$	Probability that a particle of aerodynamic diameter D_{ae} contains $\nu(D_{ae})$ agent units [0,1]
$P_i(D_{ae},Q)$	Probability that a particle with aerodynamic diameter will deposit in region i of the respiratory tract when inhaled at a rate Q where i=1: Extrathoracic region; i=2: Tracheobronchial region; i=3: Pulmonary region [0,1]
$P_A(\nu,D_{ae})$	Probability that a biological agent in a particle of size D_{ae} is active (μm)
$n(D_{ae},x,y,z,t)$	Differential particle size distribution as a function of aerodynamic diameter D_{ae} space and time ({number of particles} / {m^3} / {μm})
$P_I(\nu)$	Probability of an adverse health outcome originating in region i
dN_{Pi}	Differential probability that a particle with aerodynamic diameter D_{ae} will deposit in region i of the respiratory tract when inhaled at rate Q
dN_{Ci}	Differential number of cells that can be expected to deposit into each region i of the respiratory tract per unit volume of air inhaled if the probability that a particle of diameter D_{ae} contains ν cells (or other agent entities) is $P_\nu(D_{ae})$
dN_{Ai}	Differential number of active biological agents per unit volume of inhaled air that will deposit into region i of the respiratory tract if the probability that a cell in a particle of size D_{ae} is active as $P_A(\nu,D_{ae})$
dN_{Si}	Differential number of sites that will be produced in region i of the respiratory tract where infection may develop from deposition of a particle that contains one or more active cells per unit volume of air inhaled
dN_{Ii}	Differential probability of an adverse health outcome originating in region i of the respiratory tract

deposit in region i of the respiratory tract when inhaled at a volumetric inhalation rate Q. In the discussion that follows, we will consider separately the three deposition regions; the forms of the equations for the different regions are similar, so we will use the subscript i to denote the region of interest,

$$dN_{Pi} = P_i(D_{ae},Q)dN = P_i(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \quad (i=1,2,3) \tag{3}$$

(i=1: extrathoracic region (ET); i=2: tracheobronchial region (TB); i=3: pulmonary region (P)).

While exposure through the respiratory regions is among the most important, and is illustrated explicitly here, other modes of exposure are easily accounted for in the context of this approach. For example, many particles deposited in the ET region will be suspended in mucus and ultimately ingested, which may be the primary route of infection. The mathematical formula presented here may be modified to include this and other modes of exposure as appropriate for a given agent.

Each deposited particle may carry one or more cells, spores, virions, or other irreducible agent entities, hereafter described as cells. The total number of cells deposited into any region can be considered to be the cell dose. If the probability that a particle of diameter D_{ae} contains ν cells (or other agent entities) is, $P_\nu(D_{ae})$ the number of cells that can be expected to deposit into each region per unit volume of air inhaled is

$$dN_{Ci} = \sum_\nu \nu(D_{ae})P_\nu(D_{ae})P_i(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \quad (i=1,2,3) \tag{4}$$

$$\nu_{avg,i}D_{ae} = \sum_\nu \nu(D_{ae})P(D_{ae}). \tag{5}$$

Not all cells that deposit will be biologically active. The probability that a cell remains active may vary with particle size, time since release, temperature, relative humidity, and other factors; cells in the core of an aggregate of cells may be protected by those that surround it. Denoting the probability that a cell in a particle of size D_{ae} is active as $P_A(\nu,D_{ae})$, the number of active biological agents per unit volume of inhaled air that will deposit into region i of the respiratory tract becomes

$$dN_{Ai} = \sum_\nu \nu(D_{ae})P_\nu(D_{ae})P_A(\nu,D_{ae})P_i(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \quad (i=1,2,3). \tag{6}$$

If no agent is present in a particle, ν=0. If none of the agent is active, P_A=0. In either case, the agents may be counted by some detectors, but will not produce infection.

A deposited particle that contains at least one active agent creates a site at which infection may develop. Some agents may be sufficiently infectious that a single active cell can induce infection. In that case the probability of adverse health outcome would depend on the number of sites where any viable agent deposits. The number of such sites that will be produced in the respiratory tract where infection may develop from deposition of a particle that contains one or more active cells is, per unit volume of air inhaled,

$$dN_{Si} = \sum_{\nu\geq 1} P_\nu(D_{ae})P_A(\nu,D_{ae})P_i(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae} \quad , \quad (i=1,2,3). \tag{7}$$

The total numbers of cells deposited and potentially infectious sites created in different regions of the respiratory tract depend on the time of exposure to the agent-containing cloud and the respiration rate, and must include the contributions of all particle sizes. The resulting total number of biologically active cells deposited becomes:

$$N_{Ai} = \int_0^T Q(t)\int_0^\infty \sum_\nu \nu(D_{ae})P_\nu(D_{ae})P_A(\nu,D_{ae})P_i(D_{ae},Q)n(D_{ae},x,y,z,t)dD_{ae}dt \quad , (i=1,2,3). \tag{8}$$

Similarly, the numbers of potential infection sites created in the different regions become:

$$N_{Si} = \int_0^T Q(t) \int_0^\infty \sum_\nu P_\nu(D_{ae}) P_A(\nu, D_{ae}) P_i(D_{ae}, Q) n(D_{ae}, x, y, z, t) dD_{ae} dt \quad , (i=1,2,3). \qquad (9)$$

Health outcomes can be expressed in terms of probabilities of infection, incapacitation, or death. These depend upon the numbers of agent cells or potential infection sites that result from exposure in each of the deposition regions. We denote the probability of an adverse health outcome originating in region i as $P_i(N_{Si},N_{Ai})$. The cumulative probability of an adverse health outcome originating in *any* region of the respiratory tract, or other deposition, can be determined by considering the independent probabilities of no adverse outcomes in any of the R deposition regions, i.e.,

$$\begin{pmatrix}\text{Probability of any} \\ \text{adverse health outcome}\end{pmatrix} = 1 - \prod_{i=1}^{R} (1 - P_i). \qquad (10)$$

The foregoing analysis provides a formalism through which the hazard, measured as a probability of an adverse health outcome, can be related to measurable properties of a bioaerosol cloud, taking into account the size distribution of the aerosol particles, the variation of the composition with particle size, the behavior of particles when inhaled, and the probability that deposited particles will induce an adverse outcome. In spite of the level of detail presented, the $BAULA_{Dae}$ unit does not reach the level of representing risk as opposed to hazard. Risk will depend on the actual population exposed and, therefore, will have to include such factors as variation in deposition probability among individuals, and variations in responses of different subpopulations. Nonetheless, this analysis provides a reasonable basis for examining the suitability of monitors to assess the hazard posed by bioaerosol releases, to probe the meanings of the presently employed units of measure for bioaerosols, and to develop an evaluative framework that is applicable to all agents and all sensing technologies.

3.4 APPLICATION OF THE EQUATION TO DETECTORS

The probability of adverse health outcome is a function of the probabilities of disease initiation for each physiological region of the body. We have made the assumption that these regions are countable and finite and that disease initiation is a function of N_{Ai} and N_{Si}—the number of biological agent units (bacteria, virions, toxin units) deposited and the number of potential infection sites (particle deposited), respectively. While it would be convenient to use only N_{Ai}, there is currently insufficient scientific evidence to rule out the effect of N_{Si} in disease causation. Terms in the equations that define N_{Ai} and N_{Si} (Equations [8] and [9], respectively) include those in Box 3.3.

In theory, a detector can measure or estimate each of the terms. In practice, the detector is used to estimate specific parameters that can be extrapolated through the equation as an estimate of hazard. For example, a biological point detector can use an aerosol collector and antibody or nucleic acid identifier to estimate such actions as inhalation and uptake. The detector itself will not directly measure $BAULA_{Dae}$, rather the size and pathogen distributions in the aerosol would be linked to the best available probability distributions for the likelihood of disease. A single

point detector can determine the minimum quantity of aerosol agent material present, but unless the measurement is made proximal to the source, the detector is likely to underestimate the quantity released due to dispersion and transformation. A network of point detectors or a remote sensing system could provide more data that might integrate with meteorological measurements and algorithms for estimating transport and transformation.

BOX 3.3
Definition of variables in Equations 8 and 9

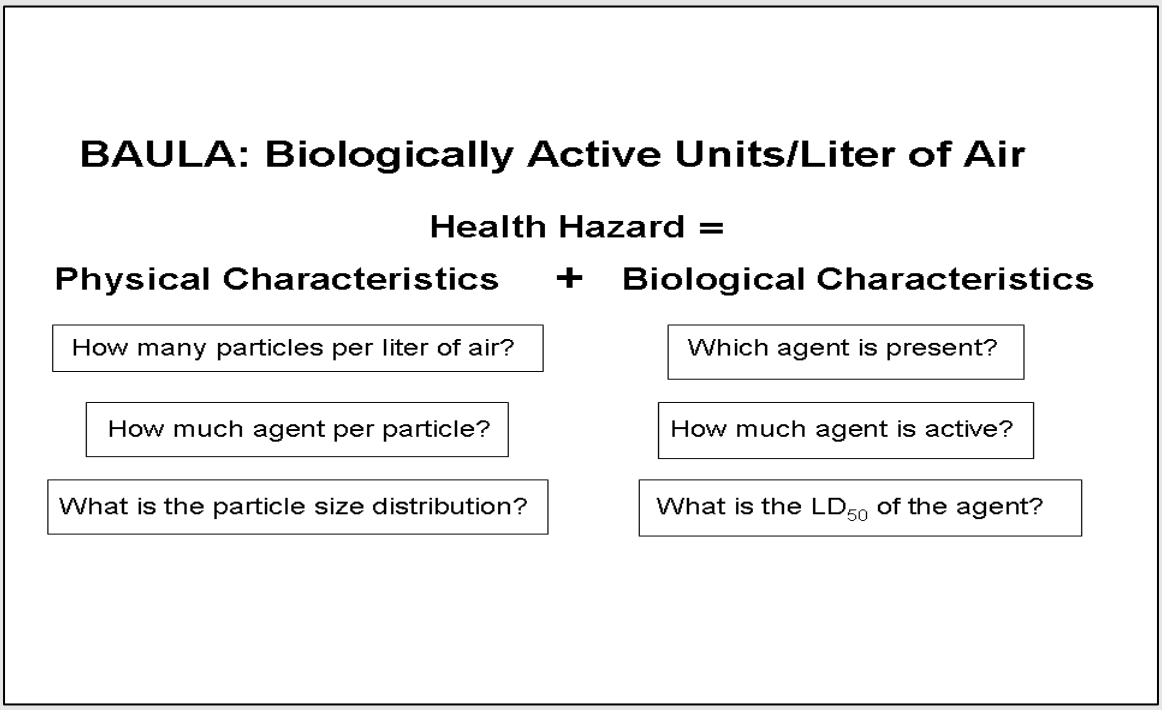
D_{ae}	Aerodynamic diameter (μm)
t	Time (min)
$Q(t)$	Volumetric inhalation rate (L/min)
$\nu(D_{ae})$	Number of agent units of aerodynamic diameter D_{ae} [0, inf)
$P\,\nu(D_{ae})$	Probability that a particle of aerodynamic diameter D_{ae} contains $\nu(D_{ae})$ agent units [0,1]
$P_i(D_{ae},Q)$	Probability that a particle with aerodynamic diameter will deposit in region i of the respiratory tract when inhaled at a rate Q where i=1: Extrathoracic region; i=2: Tracheobronchial region; i=3: Pulmonary region [0,1]
$P_A(\nu, D_{ae})$	Probability that a biological agent within a particle of size D_{ae} is active (μm)
$n(D_{ae},x,y,z,t)$	Differential particle size distribution as a function of aerodynamic diameter D_{ae}, space and time ({number of particles} / {m^3} / {μm})

In short, the detector is used to estimate the physical, temporal, and spatial parameters of the aerosol as well as provide some compositional properties or actual biological properties that are used to indicate whether the aerosol present could be hazardous or not. In a generalized perspective, the detection system is a stand-in for a human, or alternatively, a human could be regarded as a specific type of detector. In either case, detector characteristics will need to be accounted for in order to deduce the aerosol description as expressed in the equation terms described in the previous section. The mathematical description of the aerosol given above is in its most fundamental form: as an instantaneous snapshot of all space. All detection systems will have their own specific volumetric sampling rates and efficiencies as well as response times. In some cases the response times will be fast enough to follow the natural temporal variations of the aerosol size distribution and concentration so that the instrument results could be directly substituted into the formulas as presented. In other cases, the response times will be much longer and appropriate integrations of the formulas will be required to incorporate the instrument data. Finally, identification of the pathogen or toxin allows extrapolation from scientific foundations (not all of which exist today) to prediction of probable health risk. A great strength of the proposed framework is its adaptability to new developments in detector technology. As with new developments in the understanding of disease response in humans, BAULA has the flexibility to incorporate new detector capabilities into its framework. To demonstrate this flexibility, it is useful to anticipate the types of information that detectors will provide in the coming years. Box

3.4 presents four scenarios describing the data provided by a detector and the assumptions that must be made to determine $BAULA_{Dae}$ in each case.

Note that in all the scenarios listed in Box 3.4, it would be possible to determine an appropriate value of $BAULA_{Dae}$ assuming "worst-case" probabilities until the medical research and detector technology could provide more specific information.

BOX 3.4
Calculation of $BAULA_{Dae}$ with Available Data



Scenario 1
KNOWN: *Identified agent is present*
UNKNOWN: How much of the agent is active
Particle size distribution
LD_{50}

Worst case scenario: All detected agent is active
It can cause illness wherever it deposits
Agent is extremely virulent (low dose causes illness)
Calculate $BAULA_{Dae}$ assuming: 1) all detected agent is active; 2) all deposit sites have equal risk; and 3) any exposure at all is a health risk.

Consequence: Detector will sound alert for **minimum detectable concentrations of agent**

Scenario 2
KNOWN: *Identified agent is present*
How much is needed to cause disease (e.g. LD_{50})
UNKNOWN: How much of the agent is active
Particle size distribution
How much agent is needed to cause disease at a specific site of deposition

Worst case scenario: All detected agent is active
It can cause illness wherever it deposits
Calculate $BAULA_{Dae}$ assuming: 1) all agent is active; 2) all deposit sites have equal risk; and 3) correct LD_{50} value.

Consequence: Detector will sound alert if **agent concentration>LD_{50} (or other dose level, depending on specific scenario requirement)**

Scenario 3
KNOWN: *Identified agent is present*
Particle size distribution
How much is needed to cause any form of disease
How much agent is needed to cause disease at a specific site of deposition
UNKNOWN: How much of the agent is active

Worst case scenario: All detected agent is active
Calculate $BAULA_{Dae}$ assuming: 1) all detected agent is active; 2) correct risk of infection at sites where particles of the detected size range will deposit; and 3) correct LD_{50} value.

Consequence: Detector will sound alert if **agent concentration >LD_{50} AND particles are the right size to reach sites vulnerable to infection (or other dose level, depending on specific scenario requirement)**

Scenario 4
KNOWN: *Identified agent is present*
How much of the agent is active
Particle size distribution
How much is needed to cause any form of disease
How much agent is needed to cause disease at a specific site of deposition
Calculate $BAULA_{Dae}$ assuming: 1) correct proportion of agent that is active; 2) correct risk of infection at sites where particles of the detected size range will deposit; and 3) correct LD_{50} value.

Consequence: Detector will sound alert if **concentration of ACTIVE agent >LD_{50} AND particles are the right size to reach sites vulnerable to infection (or other dose level, depending on specific scenario requirement)**

3.5 COMPARISON AND CONVERSION OF $BAULA_{Dae}$ TO OTHER UNITS

As shown in Figures 3.2, 3.3, and 3.4, the unit of measurement used to describe a biological aerosol reflects the method by which a sample was collected and analyzed. Once the particles in the sample have been separated into different size ranges, the means by which they are immobilized for further analysis becomes the critical determinant of the final unit of measurement. In the case of a bacteria-containing aerosol, if the particles are impacted onto the surface of an agar plate and the number of resulting colonies is determined, the aerosol concentration will be expressed as the number of particles containing viable bacteria in each liter of air (ACPLA). If no additional samples are examined by other methods, no further description of the biological content of the aerosol is possible.

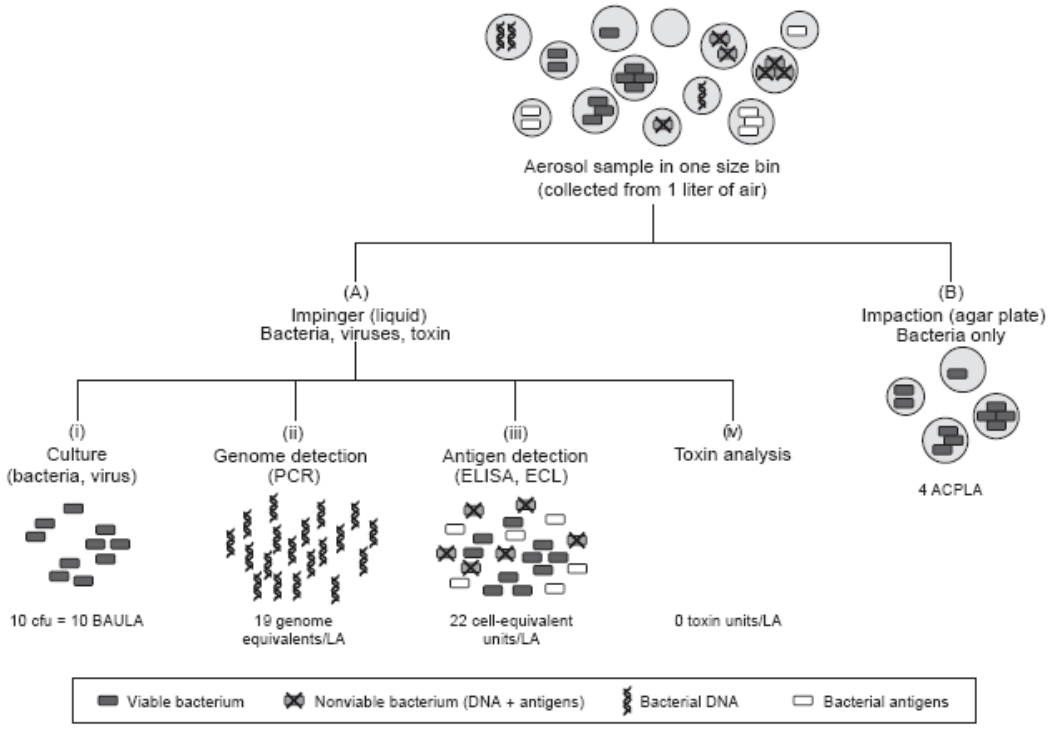


FIGURE 3.2 Methods of analyzing a bacteria-containing aerosol. If the sample is collected by impaction and culturing onto the surface of an agar plate (B), the bacterial content of the aerosol will be expressed in ACPLA. Because the particles have not been disrupted, the colony forming units only represent a count of particles with agent present. If the sample is collected into liquid (A), the unit of measure will differ depending on the subsequent analysis. In this example, the colony forming units (Ai) can be used to determine $BAULA_{Dae}$ with a known LD_{50} for the agent. For genome equivalents (Aii), the measure includes viable and nonviable bacteria and fragments of bacterial DNA in the sample. Signal contribution from antigen detection (Aiii) comes from viable and nonviable bacteria and bacterial antigens in the sample. Quantitation in *total* colony-forming units (Ai) provides the information needed to assess health hazard because it includes a measure of activity.

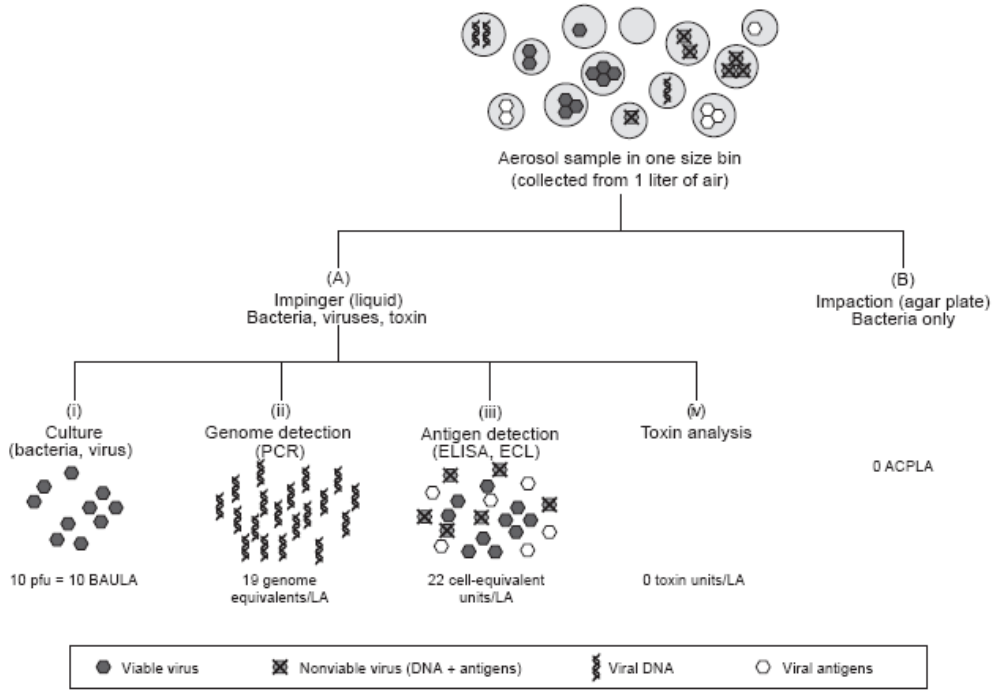


FIGURE 3.3 Methods of analyzing a virus-containing aerosol. Impaction onto an agar plate (B) is specific to bacteria and is not appropriate for viral agents. The initial sample must be collected into liquid medium (A). The subsequent analysis will determine the final unit of measure. In this example, the plaque forming units (Ai) can be used to determine $BAULA_{Dae}$ with a known LD_{50} for the agent. For genome equivalents (Aii), the measure includes viable and nonviable viruses and fragments of viral DNA in the sample. Signal contribution from antigen detection (Aiii) comes from viable and nonviable viruses and viral antigens in the sample. Quantitation in *total* plaque-forming units (Ai) provides the information needed to assess health hazard because it includes a measure of activity.

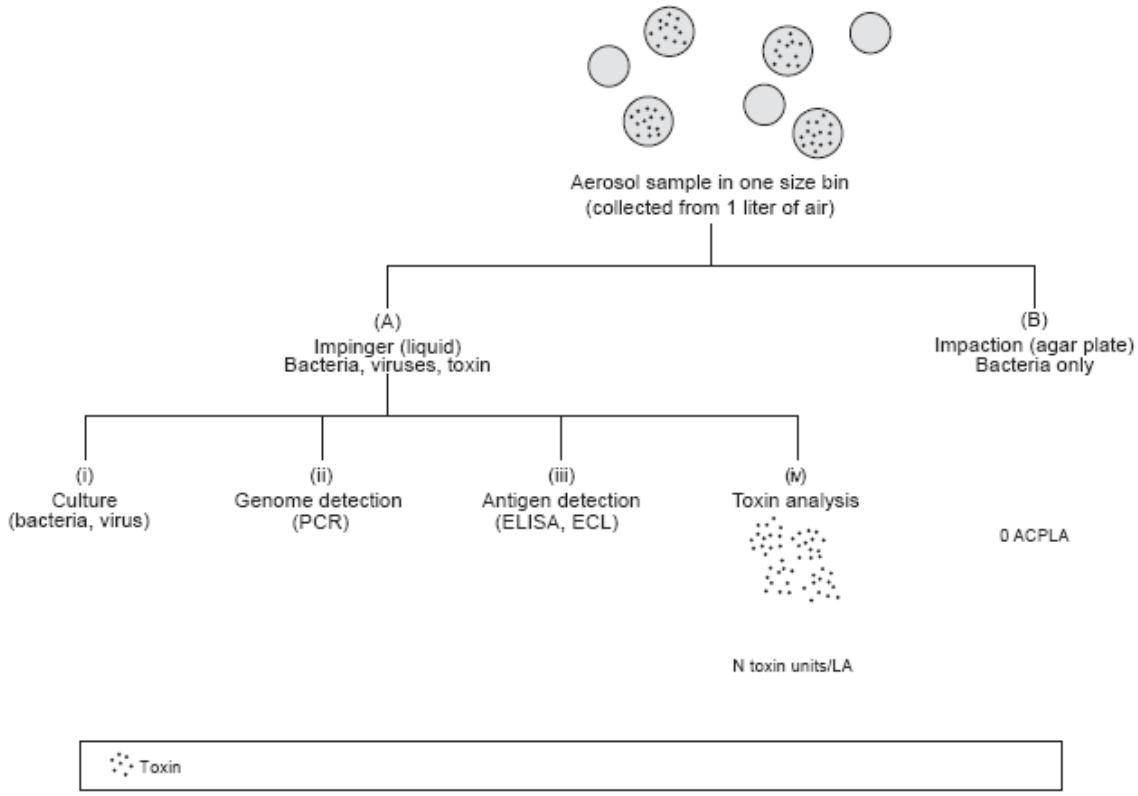


FIGURE 3.4 Method of analyzing a toxin-containing aerosol. Impaction onto an agar plate (B) is specific to bacteria and is not appropriate for toxins. The initial sample must be collected into liquid medium (A). The subsequent analysis will determine the final unit of measure.

Measurement in ACPLA provides the lower bound for estimates of the amount of viable bacteria delivered to the respiratory tract. As demonstrated in Figure 3.2, it correctly determines that dose only in the case of an aerosol of single bacteria or spores, and increasingly underestimates the delivered dose as the mean number of viable bacteria increases above one per particle. Because only bacteria are capable of replicating and producing colonies on an agar plate, this method fails to detect any viruses or toxins that may be present. ACPLA, therefore, cannot be used formally as their unit of measure. ACPLA also cannot be converted to units of mass/L, because those are only employed in quantifying certain toxins.

A main alternative to collecting particles by agar impaction is collection into a liquid medium, using an impinger, wet-walled cyclone, or other device (Figure 3.2A). Because the particles may be partially or completely disrupted as they are driven into the liquid and interact with their new environment, examination of the resulting suspension no longer permits the characterization of the original aerosol in units of ACPLA, but instead allows the determination of the total number of viable bacteria per liter of air by standard culture methods. That result, expressed in colony-forming units (CFU), is directly related to health hazard, because it indicates the dose of bacteria delivered to the respiratory tract that can replicate and cause disease. Measurement of CFU/liter of air can therefore, be converted directly to $BAULA_{Dae}$ using known

LD_{50} values (in this example, one bacterium is assumed to cause death in 50 percent of an exposed population). Except in the case of an aerosol of single spores or vegetative bacteria, the number of units of biological activity will always exceed the number of agent-containing particles (ACPLA). The same liquid suspension can also be used as the substrate for determining the total number of bacterial genome equivalents or the total quantity of a particular bacterial antigen per unit volume of air. As shown in Figure 3.2(Aii, Aiii), the number of units measured will generally be greater than the number of biologically active (viable) bacteria present. It should also be noted that it is possible to underestimate the amount of viable bacteria present if the bacteria are nonculturable on artificial laboratory media. Should these bacteria still be capable of causing infection, a measure of CFU/L could result in an underestimation of, or even complete failure to detect, health hazard.

Collection of aerosolized material into liquid medium also makes it possible to go beyond the bacterial content to characterize a number of other aspects of an aerosol. Standard culture methods can be employed to quantitate viable virus (Figure 3.3), while biological assays can be used to measure active toxin (Figure 3.4). Those results are expressed in PFU/L and toxin activity/L, respectively; and both can be used to determine $BAULA_{Dae}$. In addition, the analysis of an aliquot of the collection medium by PCR can determine the total number of genome equivalents for a specific bacterial or viral species present in a unit volume of aerosol, and an immunologic test such as ELISA, can be used to measure the amount of specific bacterial, viral, or toxin antigen. However, these methods will almost always overestimate the value of $BAULA_{Dae}$ for the same aerosol because both active and inactive agent are detected. If a portion of the aerosolized material loses viability during release and transport, but its DNA is still intact, PCR will overestimate the amount of biologically active bacteria or viruses delivered to the respiratory tract. A similar result will be obtained when antigen derived from nonviable organisms remains in aerosolized particles and is measured by an immunologic assay. The measured number of genome equivalents or quantity of antigen per liter of air should, therefore, be seen as setting an upper limit to the total amount of live viruses or bacteria that the sampled aerosol could contain, while the number of viable viruses or bacteria provides a lower bound for the total number of genomes or amount of antigen that could be present. Any attempt to convert the results from one of these detection methods to another must recognize these limitations.

Figure 3.4 shows the initial path for analyzing a sample of a toxin-containing aerosol. The initial sample must be collected into liquid medium. Testing may then be based on the biochemical characteristics of the material (mass spectroscopy), an antigen-based assay, or a direct measure of toxin activity, such as the mouse toxicity assay for botulinum toxin. The latter type of analysis will provide the most important information for determination of the predicted health effects of the aerosol.

4

Implications of BAULA for Detector Test and Evaluation

The benefits provided by the BAULA framework are dependent on its standard and consistent application to test and evaluation of detectors. As described in previous chapters, the recommended unit, $BAULA_{Dae}$, is designed to accommodate advances in both detection technologies and our understanding of the effects BWA aerosol exposures have on humans. As use of the unit adjusts to new information, it will be important to be able to relate requirements and test results to historical data. Current test and evaluation (T&E) procedures will have to be adjusted to make best use of the new unit. This chapter describes several key components of the complex T&E process and discusses how they might be affected if the BAULA framework is implemented.

4.1 THE TEST AND EVALUATION PROCESS

Separate agencies within the Department of Defense (DOD) contain the functions of (1) determination of requirements, (2) research and development, (3) procurement, and (4) test and evaluation for chemical and biological warfare defense. These agencies interact to develop, evaluate, and ultimately procure systems for BW defense. In general, the DOD development and acquisition process is divided into four stages: (1) early research and concept development, (2) technology development, (3) systems development, and (4) production and deployment. Transitions between these stages are further designated by evaluation points referred to as Milestones A, B, and C. Development across these four broad stages is further divided by eight descriptive technology readiness levels (TRLs). The transition from a developmental system to a production and procurement process is denoted by Milestone C. At this point, an independent agency must certify that the system meets the specified performance requirements before the system enters initial production and procurement. Establishment of a universal framework for health risk evaluation of BWAs will be especially useful in coordinating and aligning the activities of the various agencies and offices involved in this lengthy process.

Experimental performance tests are an integral part of several steps in the system research and development process, from early feasibility demonstration to evaluating candidate technologies. Different test facilities are used at different stages. Prior to milestone C, (BW) detection systems, or key components, are often tested with various simulants instead of live agents for safety and cost reasons. This simulant testing is typically done at test chambers and aerosol wind tunnels either at developers' facilities or sometimes at DOD-maintained test facilities (such as Dugway Proving Ground (DPG) in Utah, or Edgewood Chemical and Biological Center (ECBC) in Maryland) for critical program decision points. For more mature systems, carefully designed and controlled experiments are necessary to determine the basic performance measures: response time, probability of detection, probability of false alarm, and

minimum concentration of detection. Data from field tests, ambient breeze tunnels, and open-air sites are then related back to component tests for overall system evaluation. To expand detector evaluation capabilities, the DOD is also planning a Whole System Live Agent Testing (WSLAT) chamber to conduct live agent tests of entire systems. A brief overview of testing in laboratories, ambient breeze tunnels, and open-air sites is provided in Box 4.1.

BOX 4.1
Characteristics of Different Testing Facilities

Laboratory Testing

In the laboratory setting, aerosol wind tunnels and test chambers with different biosafety levels (BSL)(Centers for Disease Control and National Institutes of Health 2007) to accommodate simulants and live agents have been used. Laboratory experiments provide a test environment with well-controlled challenge aerosol, wind speed, wind direction, and background interferent aerosol. Depending on the sizes of the detector system and the test chamber, the whole detection system or individual components can be evaluated. A well-controlled test environment is especially useful in the initial stage of development, when laboratory tests can provide information to modify, redesign, and improve the system.

Tests in aerosol wind tunnels provide information about sampling acquisition, including the aspiration efficiency and transmission efficiency as a function of particle size, wind speed, and sampling flow rate.(Cheng et al. 2004; Cheng and Chen 2001) BSL-2 or BSL-3 test chambers enable the use of live biological agents or their simulants and are used to assess the performance of biological agent detection. In current test chambers, biological agents are usually released as a well-mixed aerosol of a constant concentration level or as spikes of agent concentration.(Jensen et al. 1992; Li and Lin 1999; Xu et al. 2003; Semler, Roth, and Semler 2004; Kesavan 2005) By varying the biological agent concentration, one can determine the minimum concentration of detection as well as response time. One can also introduce interferents into the chamber to determine discrimination capability and estimate the probability of false positive detection.

Open Air Testing

Eventually operational tests are conducted on an open air range to evaluate detector performance under real-world conditions. In these tests, simulants and agent-like organisms are released in the open air test. Active agent is never released during these tests due to the health hazard these organisms pose to the population.(National Research Council 2005) Ideally these tests would further characterize the detector's response time and false-positive detection rate in the presence of natural background. However, field tests can be time consuming and costly because of variable experimental conditions and environmental impact regulations. Highly variable environmental conditions (e.g., wind speed, wind direction, temperature, and relative humidity) affect the aerosol dispersion and introduce variability in the test data, which can require many replicate tests to average out.

Breeze Tunnels

Ambient Breeze Tunnels (ABT) provide an environment for controlled dispersion of challenge aerosols, while maintaining certain characteristics of open air conditions, including temperature, relative humidity, turbulent mixing, and even background aerosol. These tunnels are usually longer than 100 ft and have large cross-sectional areas. The wind is unidirectional, with speeds up to 5 mph. The tunnels—like a laboratory aerosol wind tunnel—have been designed to provide uniform aerosol concentration at the test section for the specified wind speed range. Because of the nature of remote detection, standoff detectors, such as LIDAR, can only be tested and evaluated in an ABT or an open air field. Both the open air field and ABT can provide performance test data on operational, full-scale detection systems.

4.2 THE CURRENT TEST AND EVALUATION PROCESS

The committee has recommended the adoption of $BAULA_{Dae}$ as part of a health risk evaluation framework because it allows cross-comparison of diverse biological agents to a single, common quality: that of health hazard. The current unit, ACPLA, requires only measurement of how many agent-containing particles are found per liter of air. $BAULA_{Dae}$ requires that several additional characteristics be considered: how much total agent is present, how much of it is active, the size distribution of the agent-containing particles, and the probability of a negative health outcome (Figure 3.1). All of these factors have an important effect on the potential of the particular agent-containing aerosol to cause illness or death. Adopting the $BAULA_{Dae}$ unit, therefore, has several implications for the T&E system. Most importantly, referee systems—the part of the test system that characterizes the conditions experienced by the detector—will have to be able to measure all the characteristics included in the $BAULA_{Dae}$ framework. Only then will referee systems be able to evaluate detectors accurately. The remainder of this chapter is devoted to considering some of these implications and describing how current test and evaluation protocols can be adapted to provide evaluation of detectors in $BAULA_{Dae}$ units. Figure 4.1 shows a generalized test system layout.

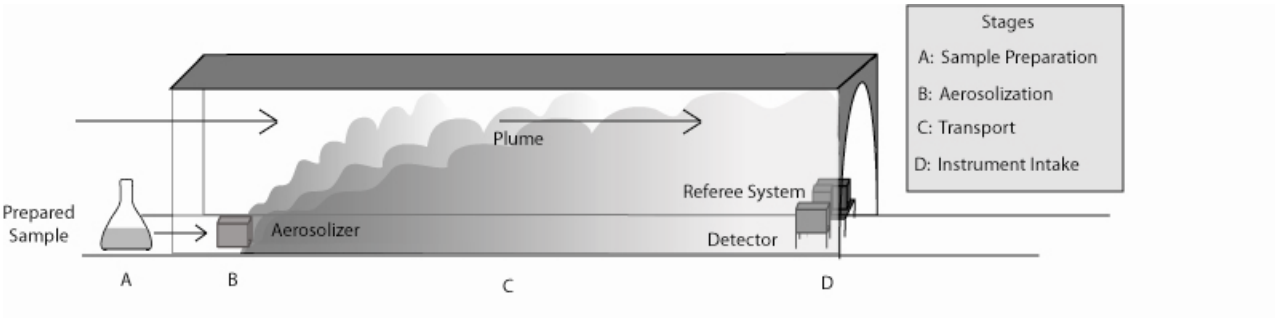


FIGURE 4.1 Sample of the agent itself or a surrogate is prepared, aerosolized, transported across the test site, and then sampled by various referee instruments. At each of these stages, the biological activity and particle size distribution may be altered.

4.2.1 Sample Preparation

An early decision in the T&E procedure is the choice and preparation of the "test material," which includes the BWA itself or a surrogate, possibly along with nonbiological particles and droplets, and interferents.

The biological test material may consist of:

- The BWA itself: Bacterial spores, vegetative bacterial cells, viruses, or toxins.
- Agent-like organisms (ALOs): Organisms having physiological, physical, and chemical properties similar to those of a corresponding BWA while presenting a reduced risk of infection. ALOs are most often derived from a vaccine or attenuated strain of a BWA, or a non-viable or inactive form of a BWA.
- Simulants: Nonpathogenic or nontoxic BWA surrogates that provide useful

evaluative information on the performace of a biodetection system and can sometimes be directly correlated with the BWA being simulated.

For example, *Bacillus subtilus var niger* (BG) spores are frequently used as a simulant for bacterial spores. *Erwinia herbicola* (EH) has been used as a simulant of vegetative bacteria, MS2 bacteriophage as a simulant of virus, and ovalbumin as a simulant of toxins. The appropriateness and utility of certain organisms as simulants is an ongoing topic of debate.

The choice of biological test material takes on a new importance if the standard of evaluation is changed from ACPLA to $BAULA_{Dae}$. Detectors being evaluated for their sensitivity in ACPLA are not currently required to distinguish between active and inactive agents, while $BAULA_{Dae}$ specifically requires detection of active agent. Furthermore, $BAULA_{Dae}$ ideally incorporates information about differences in virulence among strains of similar agents by considering the relative LD_{50} values of the strains. Thus, tests using vaccine strains or simulant may not give a direct indication of the detector's sensitivity in $BAULA_{Dae}$ units. Both cost and safety considerations affect the degree to which detectors can be tested against live, active agent—especially in an open air setting—so it is clear that future T&E systems will continue to use simulants. Over time, it is hoped that adoption of $BAULA_{Dae}$ will stimulate the development of detectors that distinguish strain differences and active from inactive agent. In the meantime, it will be important to bear in mind that detector technologies sensitive to inactive agent may have a high false positive rate when performance is measured against a referee system that measures active agent.

Because $BAULA_{Dae}$ explicitly requires distinction between active and inactive agent, sample preparation techniques will need to be standardized so that the proportion of agent killed or inactivated during the preparation process is known. Variation in test sample preparation can lead to significant differences in $BAULA_{Dae}$ values. These considerations will be particularly important in the immediate future as the majority of detectors respond to inactive agent; calibrating their sensitivity against that of the referee system will require knowledge of what proportion of the agent they are being exposed to is active.

4.2.2 Aerosolization

After the test material has been prepared, it must be aerosolized to form a "test cloud." There are both wet and dry dissemination techniques, with their own advantages and disadvantages. For the purposes of implementing $BAULA_{Dae}$, the important factors that need to be considered with respect to aerosolization are: first, the degree to which agent is inactivated during aerosolization, and, second, the particle size distribution produced by the aerosolizer. For a more detailed discussion of currently available dissemination techniques see Box 4.2.

Often test materials will work only with certain types of dissemination systems. The information on dry dissemination of biological test material is quite limited. The effects of the aerosol generation technique on the viability of the test material are largely unknown, and aerosols are generated without specific attention to how particle size distribution relates to their behavior within the respiratory tract. A biological agent or simulant may not survive the aerosolization processes because of desiccation, shear forces, or impaction of primary droplets onto the baffle design to break up large drops. Implementation of $BAULA_{Dae}$ will require that these effects be evaluated.

Box 4.2
Dissemination Techniques

Wet Dissemination Techniques

Several methods can be used to convert bulk liquid–containing biological test material–to small droplets, a process also referred to as aerosolization, atomization, nebulization, or spraying. These methods include pneumatic, or air blast, nebulization, and ultrasonic atomization.

- Air blast nebulizers(Mercer, Tillery, and Chow 1968) use compressed air to draw bulk liquid from a reservoir. The high-velocity air breaks up the liquid into droplets and then suspends the droplets as part of the aerosol. Droplets produced from this method have a volume median diameter (VMD) of 1-10 μm and a geometric standard deviation (σ_g) of 1.4-2.5.(Cheng and Chen 2001) Collison nebulizers (BGI Inc., Waltham, MA) and Hudson nebulizers (CAN Medical, Rockwall, TX) generate particles ranging in size from 0.1 to 5 μm, depending on the amounts of the test material in the aerosol suspension and operational conditions. Neither Collison nebulizers nor Hudson nebulizers are able to produce large droplets or large amount of aerosols. Agriculture sprayers (Micro Spray Ltd., Bromyar, UK) have been used successfully to produce biological simulants in open air field tests that require a high dissemination rate of droplets. The Micronair spray is vehicle mounted and uses a rotary atomization technology for droplet generation.
- Ultrasonic nebulizers atomize liquid into droplets by the mechanical energy provided from a vibrating piezoelectric crystal driven by a variable-frequency electrical oscillator. The ultrasonic nebulizer does not require a pressurized air source. Some of the problems associated with pneumatic nebulizers, including concentration and cooling effects resulting from evaporation, are not issues in ultrasonic nebulization. It is possible to produce test aerosols of different particle sizes primarily by adjusting the concentration of the suspended material in the liquid but also by adjusting the nozzle size, driving frequency of the crystal, and the liquid feed rate.

Dry Dissemination Techniques

A dry dissemination generator usually consists of a mechanism to store, transport, and deliver the test material (a powder), as well as a mechanism to disperse the powder. Powder handling systems may use a gravity feed (hopper), screw feed, rotating disks, conveyer belt or chain, brushes, or compressed cylindrical packs in which powder is delivered by scraping off the top layer.(Moss and Cheng 1989; Cheng and Chen 2001; Hinds 1999) The powder delivery rate is usually adjustable and is a key factor influencing the rate of aerosol generation and, therefore, the aerosol concentration. Two major factors influence the stability of dry powder dissemination: the feed mechanism and flow property of the powder.(Moss and Cheng 1989) Resulting aerosol size distributions depend critically on the material properties of the powder itself as well as the generation method.

Commercial and laboratory powder generators listed in Table 4.1 consist of a combination of delivery and dispersion mechanisms. These dispersion systems are usually used in the laboratory setting. For outdoor field tests, a knapsack agriculture spray, (Micro Spray Ltd., Bromyar, UK), which uses a vortex nozzle to disperse powder, has been used to disperse powder simulants.

TABLE 4.1 Dry Dissemination Methods

Product	Feeding Mechanism	Particle Size (μm)	Generation Rate (mg/min)
Wright Dust Feed	Scraping the packed plug	1-100	0.1-1000
Fluidized Bed	Chain conveyor	1-100	0.5-10
Small Scale Powder Disperser	Rotating disk	0.5-50	0.05-2.0
Jet-O-Mizer /Screw Feed	Screw feed	2-50	0.1-3
RBG 1000	Rotating brush	0.1-100	0.5-9000
GRIMM 7840	Belt conveyer	<200	15-9000
Vilnius Aerosol Generator	Rotating /Vibrating Turbine	1-50	—

4.2.3 Transport

Other potential environmental stresses on the sample are the desiccation of the bioaerosol material in transport and exposure to sunlight (UV radiation). The change in viability of the test agent between the time it leaves the aerosolizer and when it reaches the referee instruments and detector under evaluation will have to be considered.

4.2.4 Referee Instruments

The *referee system* measures and quantifies the amount of BWA aerosol in the aerosol cloud (indoors or outdoors) during a test trial. The referee system is the performance standard to which a device under test is being compared. In order to provide a reference in $BAULA_{Dae}$ units, it should provide representative and accurate information of the aerosol characteristics of the test material, including, at minimum, aerosol concentration of the active or viable agents, total aerosol concentration of viable and nonviable test material, and particle size distribution. This information is then analyzed to arrive at a standard evaluation of risk. Current referee systems have been designed to provide an accurate assessment of the ACPLA or CFU/PFU concentrations in test clouds. It should be noted that the instruments within the referee systems are not currently standardized across the T&E community. In order for these systems to provide accurate $BAULA_{Dae}$ concentration assessments, the effect of a number of steps in the evaluation process on the viability and size distribution of the particles will have to be taken into account.

The referee system may constitute a suite of instruments including:

- sample-collection devices for quantification of biological aerosol concentration and viability, and
- time-resolved measurement equipment for monitoring aerosol concentrations and particle sizes.

As detailed in Box 4.3, there are several different sampling technologies, some of which give information in near real time, and others that require laboratory follow-up to assess the biological activity of the sample. There are three types of sampling methods. *Filtration methods* allow collection of different size fractions, can sample large volumes, and can attain 100 percent gas aspiration efficiency; however these require an extraction process that decreases aerosol collection efficiency and can affect sample viability. *Impaction methods* direct particles directly onto agar plates or other culture media substrates, and the resultant colonies can be counted. Samplers are available that can provide information about the quantity of colony-forming units per unit time, as well as quantity of colony-forming units per volume of air. Samplers that can measure the aerodynamic size distribution of culturable aerosol are also available. *Liquid capture methods* (or impingers) direct particles into liquid which can be used for PCR (polymerase chain reaction), ECL, or other immunological or nucleotide-sequence-based assays with minimal extraction. Impingers are very commonly used because they are easy to use, readily available, have good collection efficiency, and are relatively low cost. However, particle size information about the sample is lost.

BOX 4.3
Referee System Sampling Techniques

Isokinetic Sampling

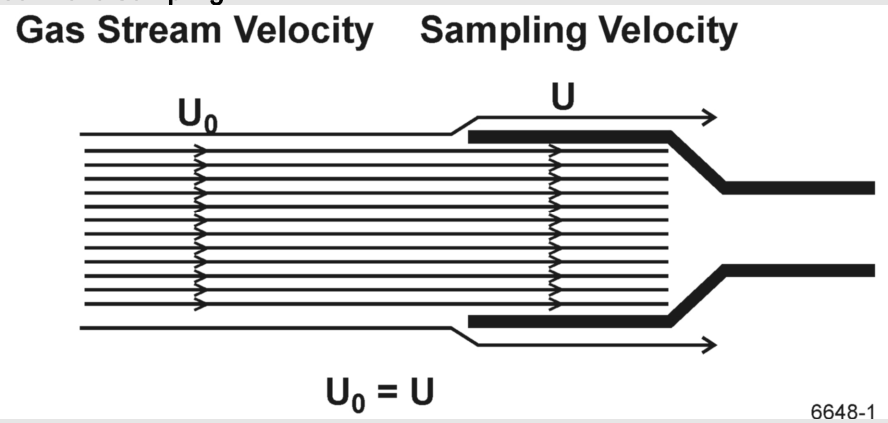


Diagram of an isokinetic filter sampler. Courtesy of Yung Sung Cheng.

Filtration

Filtration methods with adequate pore size will collect most particles larger than a minimum penetration size (typically between 0.1 to 1 μm in diameter), whereas the impaction and impingement methods will collect particles above a cutoff diameter.

Filter samplers have been used to collect test aerosol on a filter substrate to determine representative concentration of the test aerosol in the test section. There are numerous types of filtration material, but the main collection mechanism is by impaction, diffusion, interception, and electrostatic collection. The filter sample provides a time-averaged aerosol concentration. For most analytical methods,

the filter must go through an extraction process to release the particulate, which will cause a decrease in the overall collection efficiency and an increase in interferents.

In aerosol wind tunnel tests, an isokinetic filter sampler, which has 100 percent gas aspiration efficiency, is used to provide the measure of representative aerosol concentration.(Ambient Air Monitoring Reference and Equivalent Methods, Test Procedure: Full wind tunnel test. 40 CFR, 53.62, Federal Code of Regulations 1997) The isokinetic sampler shown above has an inlet sampling velocity matching the wind speed at the test section. The filter substrate, such as glass fiber filter, has near 100 percent particle removal. After the test, the filter substrate is removed for analysis to determine amounts of test material collected on the filter substrate. Usually, gravimetric analysis and analysis of fluorescent material are used to determine mass concentration (mass per volume of air) of nonbiological test material. Biological test material can be extracted into liquid medium and subsequently assayed for culturable counts and other information.

Dry filtration allows for high volume sampling which can provide for a lower detection limit. Dry filtration is the most versatile and easiest to use in a field setting, but dry filtration causes desiccation of the biological organism and, in general, is not used for methods for assessing viability.

Impaction

Slit (or slit-to-agar) samplers (New Brunswick Scientific Co, Inc., Edison, NJ) use an agar plate as the collector. The aerosol is sampled through the inlet and impacts on the agar plate through a rectangular slit nozzle. The sampling flow rate is 15 to 52 L/min controlled by a mass flow controller. The collection plate is placed on a rotating disk, which rotates at a speed from 2.0 to 99.9 minutes in a cycle. The exposed plate can be incubated, allowing viable biological particles to grow into visible colonies that can be counted. The colonies are then counted. The plate is segmented into equal time sectors, providing the information of colony-forming units as a function of sampling time. The sample volume of slit samplers is more than impingers but less than dry filtration methods.

Another sampler is the six-stage Andersen microbial impactor (Andersen Instruments, Symrna, GA). This impactor has hundreds of nozzles per stage and agar plates as collection surface. It is operated at a flow rate of 28.3 L/min. Particles are accelerated in the nozzle and those that cannot follow the flow streamline around a sharp turn make contact with the collection surface. Large particles impact on the first stage and smaller particles follow the flow until accelerated sufficiently to either impact at a later stage or pass through the system uncollected. The Andersen impactor provides bioaerosol concentration in terms of colony-forming units per unit volume air as well as aerodynamic size distribution of culturable aerosol.

Liquid Impingers

A liquid impinger may be used for biological test material that cannot be grown directly on agar plates. The liquid impinger has been widely used in test and evaluation programs to sample bacteria, spores, toxins, and viruses. A liquid impinger samples air through a nozzle and then accelerates and impinges the air jet into a collection liquid. Particles either impact directly into the liquid or are taken up by the liquid after impaction onto the bottom surface. The collected liquid sample can be used for PCR, ECL, or other established bio-assays with minimal extraction to determine the level of the specific biological agent of interest.

Several liquid impingers have been used to collect biological aerosols. An all-glass impinger draws air at 12.5/min through a 1.1-mm capillary jet. The distance between the jet orifice and the bottom of the vessel is 4 mm or 30 mm. Particle bounce, re-aerosolization, as well as liquid evaporation, have been found to have significant effects on the collection efficiency.(Grinshpun et al. 1997) A BioSampler (SKC Inc., Eighty Four, PA) is used to draw air at 12.5 L/min through three 0.75 mm nozzles. The nozzles are directed at an angle to the inner sampler wall, inducing a swirling air motion. This design minimizes particle losses after collection and has been shown to provide better collection efficiency.(Oberdorster 2000) Both impingers use about 20 mL of collection fluid.

Impingers are the most common method for quantifying viable BWA, because they are easy to use, readily available, have good collection efficiencies, and are relatively low cost. Moderate volumes can be collected, but in general they are an order of magnitude lower than the dry filtration. Impingers require monitoring of the liquid level, especially when collecting for long periods of time and in a dry climate as evaporation and re-aerosolization can become sources of error.

The assessment of biological activity that follows sampling may take hours or days; referee systems also include a time-resolved, continuous-operation component to monitor characteristics of the aerosol cloud. These near real-time measurements can be part of a feedback loop to control test aerosol concentration. Several technologies with different capabilities are available for temporal monitoring. Optical counters and aerodynamic particle-sizing instruments can measure particle concentration and size distribution (for detail on how these instruments work, their collection efficiency, and particle size detection limits, see Box 4.4). The fluorescence aerosol particle sensors use fluorescence to identify particles that may have biological origins. Some of these instruments also provide information about particle size distribution and the proportion of particles of possible biological origin in each size category. Fluorescence aerosol particle sensors can sample only a small fraction of the actual sample flow.

BOX 4.4
Time-resolved, Continuous Measurement Systems

Referee systems that collect samples provide information on biological aerosol concentration and size distribution after time-consuming laboratory assays. On the other hand, real-time measurements provide continuous monitoring of aerosol cloud and may be used to monitor aerosol in the chamber with a feedback loop to control the test aerosol concentration. It is especially useful in the laboratory chamber test to maintain a constant level of aerosol concentration or pre-set concentration profiles. Optical counters, aerodynamic particle sizers, and fluorescence aerosol particle sensors have been used in the instrument development community for rapid time-resolved measurement.

An optical particle counter (manufacturers include Climet Instruments Company, Redlands CA; Particle Measurement Systems, Boulder, CO; Grimm Technologies, Inc., Douglasville, GA) is based on the principle of light scattering. Aerosol is sampled into the instrument, typically at flow rates on the order of 1L/min. Some optical particle counters designed for measurement of relatively large (tens to hundreds of microns) particles sample at much higher flow rates. Particles are aerodynamically focused into a focal volume where they are illuminated; the light that is scattered from individual particles into a defined solid angle is measured with a photodetector. The peak pulse intensity measured with the photodetector is used to estimate particle size. That estimation requires assumptions about the particle shape and refractive index, and for the same type of particle will differ from instrument to instrument, depending on the light source employed (typically a monochromatic laser or a white light source) and the viewing angle. Particle counts are accumulated into intensity bins. The particle size distribution is inferred from the distribution of counts in those bins.

Aerodynamic particle-sizing instruments infer the aerodynamic diameters of individual aerosol particles by measuring their response to rapid changes in the velocity at which they are carried. Typically the air flow is accelerated through a nozzle to produce a high-velocity jet. Particles smaller than some threshold size will exit the nozzle at, or very close to, the gas velocity. Due to their inertia, larger particles will lag behind the gas and exit at lower velocities. The particle velocity, determined by measuring the time required for the particle to pass between two laser beams separated by a known distance, can be directly related to the aerodynamic diameter of the particle. Since aerodynamic diameter is sensitive to particle shape and orientation, sometimes correction factors may be applied to specific particle types. For example, liquid particle size measurements may be biased due to aerodynamic distortions of the droplet shape from a perfect sphere. The most common method for measurement of this kind is the **aerodynamic particle sizer** (APS) (TSI Inc., St. Paul, MN) that is based on a time-of-flight principle.(Baron, Mazumder, and Cheng 2001) The APS provides high resolution of size measurement between 0.7 and 20 μm.

The fluorescence aerosol particle sensors use fluorescence to identify particles that may have biological origins. This method, which has its roots in flow cytometry, has been applied in a number of configurations. Different instruments use different excitation wavelengths and probe different fluorescent emissions. These fluorescence measurements provide information about the possible biological content or the presence of other materials with known fluorescence characteristics.

Some instruments combine fluorescence with other measurements to provide additional information about the particles. The fluorescence aerodynamic particle sizer (FLAPS) (TSI Inc., St. Paul,

MN) integrates measurement of the fluorescence in the long and short wavelength bands that is induced by a violet (405 nm) laser diode.(Hairston, Ho, and Quant 1997) It is a single particle sensor that provides three real-time measurements of individual particles into the aerodynamic particle sizer. These measurements are the fluorescence emission intensity in two wavelengths and the scattered light intensity. The two fluorescence measurements are based on excitation illumination using a pulsed violet laser diode (405 nm) and fluorescence emission in the short and long visible wavelength bands. Due to the limited repetition rate of the laser, fluorescence is measured only for a fraction of the particles. The instrument thus reports the number distribution of particles with respect to aerodynamic diameter and the fraction of particles in the different size bins that fluoresce. The FLAPS samples aerosol at 1 L/min and detects and sizes particles in the 0.5-to-20 µm size range.

4.2.5 Validation and Calibration of the Referee System

Of critical importance to the T&E system is ensuring that the referee system can provide an accurate measure of the actual conditions at the time of the test. Currently, no instruments are available that can measure directly in $BAULA_{Dae}$ units. However, as described above, a suite of instruments can be used to measure all the factors required to evaluate $BAULA_{Dae}$. Validation and calibration of referee system performance will require taking into account earlier steps in the testing process that may have an impact on some of the qualities that use of $BAULA_{Dae}$ units require. Both the size distribution of the particles and the percentage of viable agents in the test cloud will depend on the techniques used to prepare the stock suspension, method of aerosol generation, and transport of the aerosol cloud during the test and evaluation. Considerable effort will have to be devoted to calibrating referee systems to ensure that they are measuring conditions accurately and that the measurements are comparable, especially if different test environments use different suites of instruments. For example, during the calibration phase, it will be necessary to verify such measures as:

- the proportion of sample that is active depending on different sample preparation techniques;
- the amount of sample that remains active following aerosolization;
- the size distribution of the particles leaving the entering the air to be transported;
- the amount of agent inactivated during transport from aerosol generator to the referee instruments; and
- the response function of the different sampling instruments to active and inactive agent.

These determinations will not have to be made for every test, but will have to be established for each set of instruments and for each biological agent. Careful attention to this calibration process will ensure that the $BAULA_{Dae}$ determination made by the referee system is accurate—a critical factor if it is to be used for evaluating detector performance, comparing the performance of different detectors, and comparing results from test to test and from site to site.

Sample and analysis methods for BWA materials are less mature than methods for other particulate matter, and a standard method is not currently available. BWA sampling and analysis

instruments have generally been used to assess the presence of a biological threat and are considered only semiquantitative. They are also used to evaluate the performance of laboratory and fieldable instruments that detect or identify a biological aerosol.

There are three basic types of sampling methods: filtration, impaction onto surface, and liquid capture (impingers). In order for referee systems to provide accurate assessments of $BAULA_{Dae}$, it is likely that some combination of these sampling technologies will need to be used: filtration to assess the total quantity of agent present, and impaction or impingement to assess the quantity per unit air of viable or active agent.

4.3 LIMITATIONS OF THE CURRENT SYSTEM FOR IMPLEMENTING $BAULA_{Dae}$

The previous sections summarize the variety of methods and instruments that are currently available to assess biological aerosols. There are limited standard methods to generate and characterize biological test aerosol in the test and evaluation community. The lack of standard methods and procedures already results in difficulty of translation of test results from one test bed to another and in quality control of test data. If the BAULA standard is adopted, this problem will become more severe, since more characteristics of the aerosol will need to be accurately assessed. It will be important to establish and make available consistent test protocols in order to compare the performance of different kinds of instruments. Such standard protocols will also assist in the development and improvement of bioaerosol detectors. A means to obtain consistent and available protocols is to submit the protocols through a standard development organization such as ASTM or ISO.

To implement $BAULA_{Dae}$, future tests are likely to require test aerosols with several defined-size distributions to determine the detector performance as a function of particle size. Current test aerosols do not have specified particle size distributions. As discussed in the previous section, live agents can be dispersed into aerosols with a range of particle size, depending on the formulation and dissemination technique.

Current methods to provide a measure of test aerosol have several disadvantages:

1. Filters, slit-to-agar samplers, and AGI impingers (Box 4.3) do not classify aerosol into particle size fractions and, therefore, do not provide concentration information as a function of particle size. This represents a major functional hurdle for the integration of size selective components into existing detectors.

2. Current methods provide measures of concentration of active biological material in terms of ACPLA, CFU/m^3, PFU/m^3, etc., but no information on inactive biological material. As discussed earlier, it is unlikely that test aerosol will consist only of active biological material. Most test aerosols have both active and inactive materials. Certain types of biological devices (e.g., LIDAR, fluorescence sensor, and PCR analyzer) will respond to both active and inactive biological material, but do not report the proportion of active material.

3. Referee instruments have not been well characterized in terms of their sampling efficiencies as a function of particle size, wind speed, and orientation with wind direction. Therefore, it is difficult to assess their measurement accuracy. A standard referee instrument should have near 100 percent gas sampling efficiency, as attained by the isokinetic filter samplers used for the EPA certification of particulate matter samplers or, at least, known sampling efficiencies as a function of particle size, wind speed, and wind direction. This

information is critical when particles of different sizes are included in bioaerosol sampler testing.

The variations and limitations detailed above make direct comparison of testing results from different locations or years difficult, if not impossible. Effective use of $BAULA_{Dae}$ will require a greater degree of standardization of equipment and protocols across the testing community as the unit is inherently linked to the physical properties of the aerosol cloud and particles. The committee recommends the following:

- **The method for implementing the BAULA framework should be documented, externally peer reviewed, and published.** Revisions and updates should follow similar vetting processes so that calibration, referee instruments, and testing reagents are standardized and variation is identified.
- **Aerosol challenges need to be well characterized including D_{ae}, $BAULA_{Dae}$., $BAPLA_{Dae}$, and the loss of biological activity during aerosol dissemination/transport.**
- **Biodetectors should be challenged with aerosols of defined size distributions.** At least three size distributions should be used in chamber or component testing of detectors. These should be chosen to represent deposition in the three regions of the respiratory tract to reflect health risk measurements.

4.4 IMPLEMENTING $BAULA_{DAE}$ WITH CURRENT TESTING AND REFEREE SYSTEMS

A feature of the new unit is a focus on the activity, as predicted by viability or other biological activity of interest, of the aerosol particles, and total agent contained in the particles. Currently the principal detector characteristic that is evaluated is minimum detectable concentration. Detectors are tested against lower and lower concentrations of agent until they no longer respond. In addition to determining the detector's minimum detection threshold, its response time, probability of detection, and probability of false alarm must be characterized or at least specified as part of the evaluation. One of the first objectives of a modified test procedure will be to determine the responsivity of the candidate system to one or more BW agents as reported in the appropriate unit, $BAULA_{Dae}$. Evaluating detectors for the ability to determine whether a sample is viable or nonviable, as required for measurement in the $BAULA_{Dae}$ unit, presents an interesting challenge.

In practice, it is unrealistic to expect that a pure agent material (consisting of 100 percent active agent and no other material) can be created and presented to the detection system. At least some of the agent material will be rendered nonviable and, therefore, inactive by the sample preparation, aerosolization, and transport processes. Current candidate detection systems detect both viable and nonviable agent material, but to determine a detector's sensitivity in $BAULA_{Dae}$ units, evaluators must determine the detector's limit of detection for biologically active units. In order to compare the performance of the detector to the ground truth of the referee system (which will be measuring active agent concentrations), it will be important to estimate contributions from viable agent and from previously viable agent material. Some instruments respond to material that may be present in the absence of viable as well as from previously viable target agent. For instance, optical-based detection based on aromatic amino acid (e.g., tryptophan) signatures will respond to cellular material from other species and not just from the target agent

or surrogate. These systems could indicate the presence of agent and trigger an alarm even when the referee system indicates an accurate $BAULA_{Dae}$ value of zero. Figure 4.3 gives a very simple example of how loss of activity during testing affects the assessment of detector sensitivity. More detailed examples, with suggestions on how testing in $BAULA_{Dae}$ could be implemented with current equipment despite these complications, are given in Appendix A.

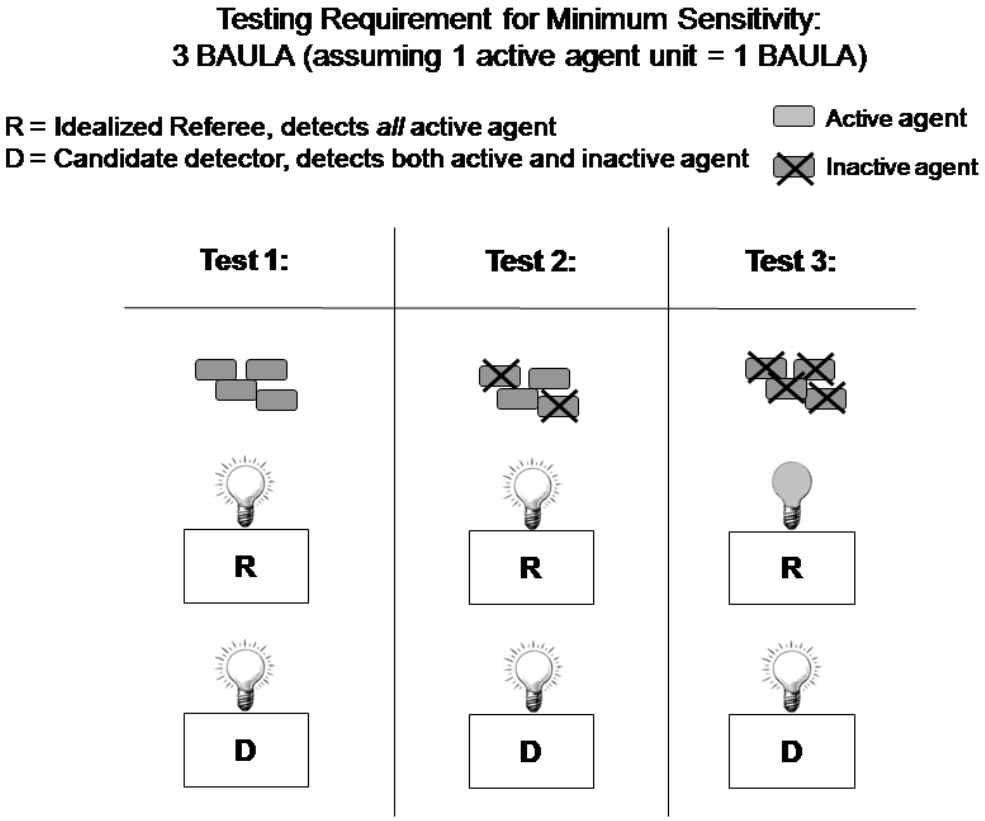


FIGURE 4.2 Testing requirement for minimum sensitivity. A simple example of the sensitivity bias that can be introduced if the response of a given detector to both active and inactive agent is not taken into account. The candidate detector will continue to signal presence of the agent even when the agent is inactive. The referee system will not signal the presence of agent as it is sensitive only to active agent.

4.5 ADDITIONAL CONSIDERATIONS

Over time it is likely to be desirable to evaluate the performance of deployed detectors and their history of signaling, either correctly or incorrectly, the presence of agent. It will be important that the test and evaluation community have enough information to modify procedures and protocols appropriately. The committee recommends that DOD **maintain the ability to learn from unanticipated events by archiving both T&E and deployed systems results (data).** Test parameters, methods, measurements, and test conditions during testing and evaluation of detectors should be recorded and archived to compare performance of deployed with tested systems. In addition, DOD should consider archiving raw data collected by deployed systems to guide future development of detectors.

5

Recommendations

A broad test and evaluation (T&E) program supports the development and deployment of biological warfare agent (BWA) detectors. From the development of testing requirements for deployment of detectors in the field, the end goal of the T&E program is to protect military personnel from BWAs and to minimize the health impact of exposure. In its current form, the T&E program relies on Agent-Containing Particles per Liter of Air (ACPLA) as the standard unit of measure of concentration for biological aerosols. This unit, while providing some mechanism for the comparison of candidate detector capabilities, does not adequately allow for conversion between various measurements or provide substantial information about the health hazard posed by detected bioaerosols. By considering the characteristics of biological aerosols that most impact the overall health risk associated with exposure, a new, robust standard framework has been developed. This new framework will have practical application for both candidate detector evaluation and a broader impact on the overall detector T&E program.

Recommendation A: **A unit of hazard should be adopted as part of the evaluative framework. The committee recommends the unit be a particle size (D_{ae}) dependent measure of biologically active units per liter of air ($BAULA_{Dae}$).**

In the context of the framework described above, a unit has been developed that describes the physical characteristics of an aerosol with the greatest impact on health outcome. A primary standard unit of biological aerosol measurement that quantifies biological activity per unit volume of air and incorporates prediction different regions of deposition sites in humans is recommended. This measure is the size-resolved number of biologically active units per liter of air ($BAULA_{Dae}$). The $BAULA_{Dae}$ unit takes into account the key information needed to estimate health hazard. Biologically active units (BAU), though measured and calculated differently for each threat agent, allow for a common measure of hazard between agents by linking the amount of active agent detected to biological activity normalized to a population activity end point (e.g., LD_{50}, ID_{50}). Incorporation of particle size into the unit accounts for the different activity levels demonstrated for particles that deposit in different regions of the respiratory tract or human body in general. When considered in the context of Recommendation A, this unit will provide baseline information about the specific hazard posed by any particular agent.

From a detector development standpoint, this unit will help direct the tailoring of detectors and requirements for specific biological agents. For example, a detector should be more sensitive to virulent agents that require less concentration to cause disease or death than for less virulent agents or those against which the at-risk population is routinely immunized.

The committee further proposes $BAPLA_{Dae}$ (size-resolved number of biologically active *particles* per liter of air) as a secondary standard unit of bioaerosol measurement to quantify the size distribution of particles in an aerosol cloud that contain biologically active material. This secondary unit of measure is recommended to provide a unit for historical comparisons with

ACPLA measurements, and also in anticipation of the possible discovery that the number of sites where aerosol particles deposit biologically active units in the respiratory tract may need to be known to predict health risk for some agents. $BAPLA_{Dae}$ would be measured in a manner similar to current methods for ACPLA, with the exception of employing a defined challenge aerosol particle size distribution and normalizing biological activity for each agent.

Recommendation B: **In support of an overall Department of Defense DOD detector evaluation philosophy that relates health risks and aerosol exposure, the committee recommends using the relationship presented in Figure 3.1 to provide a framework for relating health hazards and aerosol exposure and to identify the information provided by different types of detectors.**

This relationship, described in greater detail in Chapter 3, provides a framework for evaluation of health risk presented by exposure to aerosolized biological warfare agents. Note that, though the physical and biological characteristics of a bioaerosol are the features evaluated by a detector, the data provided by that detector cannot be appropriately assessed without consideration of the physiological effect of exposure. In addition to those specific considerations, other factors contributing to predicting health risk can be inserted into the equation to aid in the development of appropriate requirements and response protocols.

Recommendation C: **Standardize testing procedures for evaluating detectors.**

C.1 **Aerosol challenges need to be well characterized, including D_{ae}, $BAULA_{Dae}$, $BAPLA_{Dae}$, and material rendered inactive during the process of aerosol dissemination and transport.**

C.2 **Challenge biodetectors with aerosols of defined size distributions.** At least three challenge aerosols with different median aerodynamic diameters ***(D_{ae})*** should be used in chamber or component testing of detectors. These should be chosen to represent deposition in the three regions (extrathoracic, tracheobronchial, and pulmonary) of the respiratory tract.

C.3 **The standardized unit of hazard, $BAULA_{Dae}$, and the broader evaluative framework for health hazard should be adopted as DOD-wide standards, including use in T&E and procurement. The method for implementing the unit and framework should be documented, externally peer reviewed, and published.** Revisions and updates should follow similar vetting processes so that calibration, referee instruments, and testing reagents are standardized and variation is identified.

To take full advantage of the benefits provided by the new unit of hazard and the health risk evaluation framework, the T&E protocol for detector evaluation must be standardized. A full review of the testing methodology is beyond the scope of this committee. However, some issues that were brought to light during the course of this study are noted here. This list should not be considered comprehensive, but is indicative of the potential value an in-depth review could provide. For example, as $BAULA_{Dae}$ is linked to biological activity, it will be necessary to prepare test samples consistently to ensure that the amount of active agent presented to the detector does not vary across tests. It is important to understand the effect that sample preparation, aerosolization, and cloud transport have on the aerosol particles. Generated biological aerosols will change with relative humidity and temperature. These changes must be addressed to have a consistent, comparative, and scientifically rigorous test and evaluation

program. The effect of desiccation of the biological material with propagation will change the aerodynamic properties of the particles as well as the biological properties and must be taken into account. The referee instruments will need to be carefully calibrated and chosen to enable characterization of particle size and the proportion of active agent in the aerosol.

Effective use of $BAULA_{Dae}$ and the broader risk framework will require coordination between the various areas of the DOD test and evaluation community, as it is critically linked with the health outcome resulting from exposure. This will impact the development of detector requirements and will require input from, and coordination with, the biological testing laboratories.

Recommendation D: **Survey the literature to better understand the transport and inhalation of particles to improve selection of appropriate D_{ae} (median aerodynamic diameter) challenge aerosols for test and evaluation. Balance this knowledge with intelligence of possible threats.** Specifically examine whether to include testing of particles smaller than 1μm and larger than 10μm.

The site of deposition of a particle with active agent in the respiratory tract has a significant impact on the final health outcome of exposure. As the body of research on the transport and inhalation of particles continues to grow, it should be reflected in the selection of particle size distributions that best represent the potential health threat in the field.

Recommendation E: **Maintain ability to learn from unanticipated events by archiving data.**

E.1. **Archive parameters, methods, measurements, and test conditions during T&E of detectors.**

E.2. **Consider archiving raw data collected by deployed systems to guide future development of detectors through performance evaluation.**

Once a detector has been deployed in the field, it can still provide important information to aid in the development of future detector technologies and refinement of the application of $BAULA_{Dae}$. By archiving raw data received by deployed detectors, it is possible to compare data from true or false positives with the data received in the test chamber. In addition, it is likely that the parameters used to calculate $BAULA_{Dae}$ will change over time for specific agents as more clinical data about the physiological effects of exposure are acquired. A data archive of test parameters, methods, measurements, and test conditions will make it possible to compare datasets acquired at different times and places.

Recommendation F.1: **For priority DOD scenarios, an evaluation of uncertainty in the science, measurements, and situational awareness should be conducted so that resources can be invested in reducing the largest uncertainties that impact decision making.**

The data matrix required to make $BAULA_{Dae}$ maximally useful is quite large and acquiring all pertinent data about the health effects of size-resolved particles for every strain and variant of agent is not feasible. As a result it will be necessary to periodically review and prioritize the allocation of resources so that they may be invested in reducing the largest uncertainties that impact decision making.

Recommendation F.2: **An applied science program should be designed and executed to obtain information that will both improve the accuracy of the terms of the equation and help evaluate threats and their inherent uncertainties.** This will require information about different biothreat agents, instrumentation types, and scenarios important to DOD. The information will be of value to biodetector testing and many other aspects of the overall military biodefense program.

An applied science program should be created to act on the priorities as they become apparent. For example, as intelligence is gathered, it may become apparent that one type of agent is a greater threat than another. In this case, a program to investigate in detail the effect of particle size on health outcome for that strain or agent type may be in order. An effective applied science program would enable a quick response to emerging threats and any new data.

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APPENDIXES

A

Evaluating Detector Signals

The tester has access to challenge material, limited information about the test instrument such as inlet flow rate, power dissipation, etc. (in order to not restrict innovation and progress, the test instrument is treated as a "black box" as much as possible), and the response of the test instrument to the challenge. The test protocols allow for the detector to produce only a binary response. If agent is detected, a positive response is recorded; if no agent is detected, a negative response is recorded. For instance, in a series of *N* aerosol challenge experiments, the instrument response R is recorded as shown in Table A.1.

TABLE A.1 Experimental Protocol for Determining a Detector's Sensitivity Threshold

Experiment	Response R	Comments
1	1	Positive – agent detected by the instrument
2	1	Positive
…		
i	1	Positive
i+1	0	Negative – agent not detected by the instrument
…		
N	0	Negative

Historically a the progression of test experiments began with a concentration, C, of target material that was then diluted until the instrument no longer detected the material—usually done by comparison of a measurement to a threshold. In our example, this occurs at experiment *i*, and we designate $C_{threshold} = C_i$ the lowest target concentration to produce a positive detection. If the only material present in the challenge were viable agent target, a measurement in CFU, PFU, and animal toxicity testing (for bacteria, viruses, and toxins, respectively), for $C_{threshold}$ could be converted into BAULA units, and this would be the minimum detectable concentration of the instrument in BAULA.

In the section, this simplified illustration of instrument response as a function of target concentration will be exploited to discuss aspects of performance testing for determining minimum detectable concentration in terms of BAULA units. We acknowledge here that in practice instrument response, R(c), will not have a sharp step-function in target concentration in which the detected quantity generates a robust positive signal down to some threshold value and then indicates a null detection below that value. More typically, R(c) will drop off over some extended range of concentration values with more of an "S" shaped response function. For instruments that have a binary "target present/target absent" response this means that the point of transition will fluctuate in a statistical sense over some range of target concentration. For example, if the trial illustrated in Table A.1. were repeated a number of times the transition point

represented by the $i^{th}+1$ concentration would have some variance, meaning that on some tests the transition may occur at the i^{th} or $i^{th}+2$ absolute target concentrations. More importantly, for any given test, the transition may not be monotonic but could indicate a negative response on the i^{th} concentration followed by a positive response on the $i^{th}+1$ or lower concentration. The essence of this behavior is the concept of probability of detection. In general, for any fixed absolute target concentration, a large number of tests would yield a certain percentage of positives and a complementary number of negatives. In the limit of the number of tests becoming very large, we assign the percentage of positive responses for actual target concentrations as the probability of detection (P_D). Therefore threshold concentration which is designated as the minimum detectable concentration must be selected on the basis of what P_D is desired as an operational point. The two remaining fundamental metrics of a sensor system: (a) response time (integration time) and (b) probability of false positive are also interconnected to the determination of a minimum detectable threshold. In general, these four quantities are functionally related and it is not possible to specify one without also specifying the others. A complete outline of a test and evaluation procedure for determining the functional relationships of these four metrics is beyond the scope of this report. We continue this section under the assumptions that the aerosol challenge will be presented to the sensor being evaluated for a period of time consistent with the required response time, that the determination of the minimum concentration threshold is made in the absence of deliberate "confounder" or "interferent" materials, and that the detector response as a function of target concentration is sufficiently sharp to designate a reasonably repeatable and meaningful threshold concentration without having to define a precise P_D.

As was mentioned earlier, for instruments that are responsive to both viable and nonviable target agent (e.g., PCR, antibody capture assays, etc.), the estimate of test instrument detection threshold based on CFU, PFU, and animal toxicity testing in the referee system might be overly optimistic. To make this point more concretely, we provide an example where the referee system estimates both viable agent and previously viable agent in the challenge material. We then generalize to any number of parameters in the challenge that are under the control of the test. In both cases, we include the effect due to a background noise concentration and construct a mathematical approach to estimate the test instrument's minimum detectable concentration.

Consider a procedure for determining a test instrument's detection threshold in BAULA consisting of:

- an idealized referee system that accurately measures active agent in a sample;
- a test instrument that uses a nucleic acid detection scheme that accurately senses the number of genome equivalent (GE) copies of the target agent to determine agent concentration with an inherent detection limit of 20 GE; and
- a target agent pathogenic enough that 1 active organism or molecule exposure or dosage is the LD_{50}.

As the goal of the test is to determine the test detector's sensitivity in BAULA, all data received from both the referee and test systems are converted to BAULA. (1 Referee unit converting to 1 BAULA; 1 GE of the test detector converting to 1 BAULA) In this scenario, on reaching the referee and test instrument, the challenge material has a concentration A of viable target agent and I of target agent that has degraded to become inactive. A series of dilution experiments are performed to test the detection threshold of the instrument. Assume a situation (Case 1) where there is twice as much active agent as inactive agent as in Table A.2. If the test instrument were

only sensitive to active agent, the limit of detection would have been determined in experiment dilution number 2, with a BAULA of 32 instead of the reported 48. Because the referee sees only BAULA, we would have empirically estimated the limit of detection to be 16 at dilution 3. Repeating the experiments will not remove this inherent measurement bias.

TABLE A.2 Effect of detection of inactive sample on BAULA determination: Case 1, 1:2 Ratio of Inactive to Active Agent

Experiment Number	**True Concentration (per liter of air) of Agent at Location of Referee and Instrument**		**Concentration in BAULA Determined by Referee Measurements (detects only active agent)**	**Concentration in BAULA Determined by Test Instrument Measurements (detects both active and inactive agent in GE)**	**Response of Test Detector**
	Active agent (*A*)	Inactive agent (*I*)			
0	128	64	128	192	Positive
1	64	32	64	96	Positive
2	32	16	32	48	Positive
3	16	8	16	24	Positive
4	8	4	8	12	Negative (beyond instrument sensitivity of 20 GE)
5	4	2	4	6	Negative
6	2	1	2	3	Negative

Table A.3 describes test Case 2 where twice as much inactive agent as active agent is presented to the referee and the test instrument. In this experiment, when the referee is measuring 32 BAULA, the instrument is actually exploiting 96 GE-based BAULA. Assuming that the intrinsic GE copy detection capability is 20, then the sensor limit of detection would be empirically estimated to be 8 BAULA. In summary, as we decrease the ratio of inactive target agent to active agent from 1:2 in Case 1 to 2:1 in Case 2, the instrument's apparent minimum detectable concentration in BAULA, as experimentally estimated by a perfect referee system at the location of the test instrument, goes from 16 to 8.

TABLE A.3 Effect of detection of inactive agent on BAULA calculation: Case 2, 2:1 Ratio of Inactive to Active Agent

Experiment Number	**True Concentration (per liter of air) of Agent at Location of Referee and Instrument**		**Concentration in BAULA Determined by Referee Measurements (detects only active agent)**	**Concentration in BAULA Determined by Test Instrument Measurements (detects both active and inactive agent in GE)**	**Response of Test Detector**
	Active agent (*A*)	Inactive agent (*I*)			
0	128	256	128	384	Positive
1	64	128	64	192	Positive
2	32	64	32	96	Positive
3	16	32	16	48	Positive
4	8	16	8	24	Positive
5	4	8	4	12	Negative (beyond instrument sensitivity of 20 GE)
6	2	4	2	6	Negative

It is apparent from examining the tables that an estimate of GE copies (and conversion to BAULA) by the referee can be used to correct the bias. We introduce a matrix construct to help generalize to the different instruments that DOD currently and will potentially test.

For a specific target agent define three variables:

- A as the concentration of active agent at the referee (and test instrument), using BAULA or an appropriate surrogate (e.g., infectious unit or toxic unit);
- I as the concentration of previously active agent (i.e., inactive agent) that can be used to infer what BAULA likely existed before inactivation; and
- N as the effective concentration of material that is noise to the instrument. This term captures noise as well as signal contributions from any components of the measurement technology in the test instrument that are not described by the other terms of the referee system. It is not related to parameters considered relevant to sensitivity of the test instrument or relevant to defining the target agent.

In practice A is estimated using BAULA or surrogates for BAULA (e.g., CFU), I is estimated using genome copy (e.g., to estimate the number of active and inactive bacteria or viruses and remove the contribution from active agent using the estimate for A). The random variable N is not estimated by the referee system as defined. The response of the test instrument to changes in A , I, and N may be complex. Since we are seeking to find a minimum detectable concentration threshold, we make a simplifying assumption that near the minimum concentration value the three component variables contribute with linear (but unknown) weights. For a specific experiment "k" where the transition from positive to negative detection occurs, $aA_k + iI_k + nN_k = \Lambda$, where Λ is the threshold of the test instrument in BAULA-equivalent units. Recall that $C_{threshold}$ (see Table A.1) was selected by diluting concentration until the test instrument no

longer triggered.

A second series of experiments can be conducted where the starting material is first diluted to a different ratio of active to inactive agent. Care must be taken to ensure that the ratios of active to inactive agent are randomized. For instance, dilution of the k^{th} experiment by inactivating an aliquot fraction p of A_k would unfortunately result in a series of experiments given by

$$a(1\text{-}p)A_k + i(pA_k + I_k) + nN_k = \Lambda$$

that are insufficient to uniquely determine a, i, and n.

We can use control of the source material to vary the A_k / I_k ratio and the dilution protocol described in Tables A.2 and A.3 above to determine the instrument sensitivity with the referee estimates for active agent, A_k, and inactivated agent, I_k. By definition the referee does not estimate n or N_k. The collection of experiments can be represented in matrix form as:

$$\begin{bmatrix} \dots & & \\ A_k & I_k & 1 \\ & & \dots \end{bmatrix} \begin{bmatrix} a \\ i \\ nN_k \end{bmatrix} = \begin{bmatrix} \dots \\ \Lambda_k \\ \dots \end{bmatrix}.$$

When the challenge material is composed of viable agent only, I_k=0. The referee system estimates $<A_k>$ and, therefore, the matrix reduces to one experiment:

$$aA_k + nN_k = \Lambda_k$$

As expected, the minimum concentration threshold is biased by a noise term nN_k, and scaled by a, which can be interpreted as an instrument efficiency parameter. Since the noise term cannot be removed without a second experiment, it is necessary to change the A_k / I_k ratio and repeat the series of dilutions for the new A_k concentration of target agent and with nonzero inactive component I_k. We will now need to estimate the parameter i, in order to accurately estimate the detection threshold. We experimentally estimate A_k, I_k, and are given Λ_k by the test instrument as the estimated BAULA limit of detection.

To solve for a, i, and nN_k, we need three experiments that result in linearly independent rows in the matrix equation. A series of three experiments beginning with a range of A_k and I_k is sufficient to solve for a, i, and nN_k uniquely. Using this series for an idealized noise-free example with the GE detector, our equations become

$$\begin{bmatrix} 16 & 16 & 1 \\ 8 & 16 & 1 \\ 0 & 32 & 1 \end{bmatrix} \begin{bmatrix} a \\ i \\ nN_k \end{bmatrix} = \begin{bmatrix} 32 \\ 24 \\ 32 \end{bmatrix}.$$

Because this is an example, the matrix was selected to have an inverse.

$$\begin{bmatrix} \frac{1}{8} & \frac{-1}{8} & 0 \\ \frac{1}{16} & \frac{-1}{8} & \frac{1}{16} \\ -2 & 4 & -1 \end{bmatrix}.$$

Solving, we determine a=1, i=1, and nN_k=0.

For data with a nonzero bias term or noise (i.e., where the three equations do not intersect at a single point), additional experiments can be conducted and a least squares approach used to solve

$$\begin{bmatrix} a \\ i \\ nN_k \end{bmatrix} = \left(M^T M\right)^{-1} M^T \begin{bmatrix} \dots \\ \Lambda_k \\ \dots \end{bmatrix},$$

where $M = \begin{bmatrix} \dots & & \\ A_k & I_k & 1 \\ & & \dots \end{bmatrix}$ and M^T and $(M^TM)^{-1}$ are the matrix transpose of M and left inverse of M^TM, respectively.

If desired, additional columns can be added to M for each signature considered acceptable or unacceptable to contribute to estimation of sensitivity of the instrument. Just as we added previously active agent and compensated for it, we could add columns that represent optical cross-section for a specific wavelength of light, antigen binding, etc. Basically any parameter that the referee estimates can be introduced into the matrix to organize calculation of the limit of detection. With the parameters estimated the tester can decide whether the components due to non-BAULA contributions are appropriate test surrogates or are appropriate contributors to positive detection.

It should be emphasized that the test instrument response R(A, I, N) is most likely a complex function of the parameters that we have been discussing. Our representation of a linear combination of the parameters can be considered a low order approximation to R(A, I, N) or as a linearized estimate of the optimization search near the limit of detection. In either case it is an approximation. The measurement that is closest to the BAULA-defined limit of detection is the one that is often the most difficult or impossible—100% biologically active agent in the challenge with no material that interferes with the test instrument or the referee. As the test arrangement deviates from this challenge, the matrix formulation becomes a method to track contributions from BAULA and non-BAULA terms as well as an overall estimate of the noise. Selecting the most significant non-BAULA contributions is important to an accurate estimate of sensitivity in BAULA units.

Consider Table A.4 where the test instrument uses GE to estimate BAULA, with the additional complexity that the test instrument is not as specific as the referee and it, therefore, senses the equivalent of 25 BAULA from a near neighbor organism that contaminated the challenge and is no longer viable. Repeating the experiment from Table A.3:

TABLE A.4 Effect of detection of inactive agent on BAULA calculation: Case 3, Test Sample Contamination with Near Neighbor Organism

Experiment Number	**True Concentration (per liter of air) of Agent at Location of Referee and Instrument**		**Concentration in BAULA Determined by Referee Measurements (detects only active agent)**	**Concentration in BAULA Determined by Test Instrument Measurements (detects both active and inactive agent and contaminant in GE)**	**Response of Test Detector**
	Active agent (*A*)	Inactive agent (*I*)			
0	128	256	128	394	Positive
1	64	128	64	202	Positive
2	32	64	32	106	Positive
3	16	32	16	58	Positive
4	8	16	8	34	Positive
5	4	8	4	22	Positive
6	2	4	2	16	Negative (beyond instrument sensitivity of 20 GE)

As before, a series of three experiments is sufficient to solve for a, i, and nN_k uniquely. Using this series for the GE test instrument that is biased by a near neighbor contaminant (Λ is increased by 10 in each experiment), our equations become

$$\begin{bmatrix} 16 & 16 & 1 \\ 8 & 16 & 1 \\ 0 & 32 & 1 \end{bmatrix} \begin{bmatrix} a \\ i \\ nN_k \end{bmatrix} = \begin{bmatrix} 42 \\ 34 \\ 42 \end{bmatrix}.$$

We use $(M^TM)^{-1}$ from before to solve (in a least squares sense) a=1, i=1, and nN_k=10.

B

Statement of Task

At the request of the U.S. Army, the National Academies will conduct a study that will address the use of measurement in the testing of aerosol detectors. The U.S. Army's current requirements for evaluating aerosol detectors are stated in ACPLA or Agent Containing Particles per Liter of Air. However, there is not an adequate mechanism for determining if equipment meets the standard. The Army seeks a standard unit of measure that can be used for biological material independent of the state of the material (aerosol or aerosol resuspended in liquid) and independent of agent type (bacteria, viruses, or toxins).
The study will specifically address the following questions:

- Is there a single unit of measure that is appropriate for use in the evaluation of aerosol detectors?
- What are the possible alternatives to the use of ACPLA and what are the advantages and disadvantages of their use?
- Are different measures appropriate in different circumstances?
- Is there a robust way to convert between various units of measure?

C

Committee Member Biographies

J. Patrick Fitch (CHAIR), National Biodefense Analysis and Countermeasures Center

Dr. Fitch is laboratory director of NBACC and president of the Battelle National Biodefense Institute, LLC (BNBI). NBACC is managed and operated by BNBI as a federally funded research and development center for the Department of Homeland Security. Dr. Fitch has a background in genomics, bioengineering, and electrical engineering. He has also served as Lawrence Livermore National Laboratory (LLNL) program leader for chemical and biological national security; division leader for engineering research, bioengineering, and genomics; and LLNL director of the Center for Healthcare Technologies. Dr. Fitch holds a Ph.D. in electrical engineering from Purdue University and B.S. degrees in physics and engineering sciences from Loyola College. He chaired the National Research Council Committee on Review of Testing and Evaluation Methodology for Biological Point Detectors and served on the Committee for an Assessment of Naval Forces' Defense Capabilities Against Chemical and Biological Warfare Threats and is a member of the Institute of Medicine Forum on Microbial Threats.

Mike Bray, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Dr. Bray is currently a medical officer in the Biodefense Clinical Research Branch, Division of Clinical Research, NIAID. He is a physician and virologist, and is board-certified in anatomic and forensic pathology, specializing in the control of hazardous viruses and defense against biological weapons. His expertise is in the area of virus research under BSL-2, -3, and -4 containment, and he is the author or coauthor of some 100 research papers and review articles in pathology, virology and biodefense. Dr. Bray received a bachelor of science degree from the University of Oregon, an M.D. degree with honors from Dartmouth Medical School, and an M.P.H. degree from Johns Hopkins University.

Yung-Sung Cheng, Lovelace Respiratory Research Institute

Dr. Cheng is the director of the Aerosol and Respiratory Dosimetry Program and Inhalation Drug Delivery Center at Lovelace Respiratory Research Institute. Dr. Cheng is particularly interested in characteristics of airborne material that will influence its transport, collection, deposition, and retention in humans and environments. He has been heavily involved in the design and operation of high-quality systems for animal inhalation exposure studies. More recently he has been involved in design and evaluation of air sampling instruments for radioactive aerosol and biological agents and wind tunnel testing of air sampling instruments. He has published over 200 papers in the aerosol-related research areas. Dr. Cheng received his Ph.D. in chemical engineering from Syracuse University.

Kathryn L. Creek, Los Alamos National Laboratory

Ms. Creek is currently program manager for L-3 Communications, Applied Technologies Division, where she focuses on the commercial development of decontamination technologies. Previously she was project leader and program manager of the Los Alamos National Security Applied Modern Physics Group. The goal of one of her current projects is to enhance and complement development of detection and analysis protocols by providing essential information on the background microbial populations in public settings. Over the period of a week environmental conditions were monitored, employing fluorescence based bioaerosol triggers, particle counters and sizers, and other standard sampling methods commonly used in environmental analysis of air quality. In addition to these standard methods of analysis, methods were used to provide for a more in-depth understanding of the biotriggers' response to typical air contaminants present in public facilities. Ms. Creek has over 15 years of experience as an aerosol scientist and industrial hygienist. She received her M.S. in industrial hygiene from the University of Oklahoma.

Jay Eversole, Naval Research Laboratory

Dr. Eversole is currently a research physicist at the Optical Sciences Division of the Naval Research Laboratory (NRL), Washington, DC, where he is head of the aerosol physics section primarily aimed at bioaerosol detection development. Dr. Eversole received his doctorate in physics in 1975 from the University of California, Santa Barbara. Following two years as a National Research Council postdoctoral fellow, he worked for the University of Dayton Research Institute as an on-site research scientist at the Astrophysics Laboratory, Edwards Air Force Base, CA, in the area of optical combustion diagnostic measurements, especially for aerosol-laden exhaust gasses. Dr. Eversole joined NRL in 1985, has co-authored over 50 refereed research journal articles on various aspects of the optical properties of particulate materials, and single liquid droplets.

Richard C. Flagan, California Institute of Technology

Dr. Flagan is the Irma and Ross McCollum/William H. Corcoran Professor of Chemical Engineering and Professor of Environmental Science and Engineering at the California Institute of Technology. He also serves as the executive officer for the Department of Chemical Engineering. He received his B.S. in mechanical engineering from the University of Michigan in 1969, and his Ph.D. in mechanical engineering from M.I.T. in 1973. He joined the Caltech faculty of Environmental Engineering Science in 1975. Since joining the faculty at Caltech, his research has focused on aerosols, beginning with the study of nanoparticle formation during pulverized coal combustion, and gradually migrating toward atmospheric aerosols and applications of methods of aerosol science to the development of new materials and nanoparticle-based microelectronic and photonic devices. To better accommodate the increasing chemical process component of his research, Dr. Flagan moved to the Department of Chemical Engineering in 1990. He continues research in both atmospheric aerosols and aerosol nanotechnology. Dr. Flagan has received a number of awards in honor of his work in these areas. He is an active member of the American Association for Aerosol Research, having served as its treasurer, president, and presently, as the editor in chief of its journal, *Aerosol Science and Technology*.

Michael R. Kuhlman, Battelle National Biodefense Institute

Dr. Kuhlman is director of the National Biological Threat Characterization Center of the Department of Homeland Security, National Biodefense Analysis and Countermeasures Center. Formerly he was manager of Battelle's Aerosol and Process Technologies (APT) product line, a multidisciplinary technical expertise focused on problems associated with airborne hazards: their production, propagation, detection, and mitigation. In this position he manages over 100 research staff members, and is responsible for state-of-the-art aerosol research laboratories. Throughout most of his 25-year career with Battelle, Dr. Kuhlman has been responsible for the performance and technical oversight of research programs involving various aspects of aerosol science, ranging from the dynamics of aerosol particles to the generation, collection, and detection of biological aerosols. The applications of this work have ranged from nuclear safety to atmospheric chemistry to the technical assessment of various respirable hazards. Dr. Kulman received his Ph.D. in environmental science and engineering from the University of North Carolina.

Stanley Maloy, San Diego State University

Dr. Maloy is the dean of the College of Sciences and the director of the Center for Microbial Sciences at San Diego State University. He has extensive experience in the area of bacteria pathogenesis, particularly salmonella. Dr. Maloy obtained his Ph.D. in molecular biology and biochemistry from the University of California, Irvine.

Gary Resnick, Los Alamos National Laboratory

Dr. Resnick is the Bioscience Division leader at Los Alamos National Laboratory. He is an internationally recognized scientist in the area of chemical and biological defense, with extensive leadership and management experience. His scientific and technical accomplishments encompass all aspects of research, development, and testing of chemical warfare agents and chemical and biological defense systems. In addition, he has been an active member of the interagency and international chemical and biological arms control communities. Previous positions held include: associate center director for chemical and biological (CB) defense, Center for Homeland Security at Los Alamos National Laboratory; director of CB Defense, Defense Threat Reduction Agency; director of research and technology, Edgewood Chemical and Biological Center; technical director, U.S. Army Dugway Proving Ground, and staff scientist at the U.S. Environmental Protection Agency. He holds a B.S. degree from Cornell University, an M.S. degree from Long Island University, and a Ph.D. in microbiology from the University of Rhode Island.

D

Acronyms and Abbreviations

ABT – Ambient breeze tunnel
ACPLA – Agent-Containing Particles per Liter of Air
AGI – All-glass impinger
ALO – Agent-like organism
APS – Aerodynamic particle sizer
ASTM – American Society for Testing and Materials
BAP – Biologically active aerosol particles
$BAPLA_{Dae}$ – Biologically active aerosol particles per liter of air size resolved by aerodynamic diameter
BAU – Biologically active units
$BAULA_{Dae}$ – Biologically active units per liter of air size resolved by aerodynamic diameter
BAWS – Biological agent warning sensor
BSL – Biosafety level
BW – Biological warfare
BWA – Biological warfare agent
CFU – Colony-forming units
D_{ae} – Aerodynamic diameter
DARPA – Defense Advanced Research Projects Agency
DHS – Department of Homeland Security
DNA – Deoxyribonucleic acid
DOD – Department of Defense
DPG – Dugway Proving Ground
DTRA – Defense Threat Reduction Agency
ECBC – Edgewood Chemical and Biological Center
ECL – Electrochemiluminescence
ELISA – Enzyme-linked immunosorbent assay
ET – Extrathoracic region of the respiratory tract
FLAPS – Fluorescence aerodynamic particle sizer
GE – Genomic equivalents
ID_{50} – The dosage that is required to infect 50 percent of a given population
JPEO –Joint Program Executive Office for Chemical and Biological Defense
JRO – Joint Requirements Office
JT&E – Joint test and evaluation
LA – Liter of air
LD_{50} – The dosage that is lethal to 50 percent of a given population
LOD – Limit of detection

MMAD – Mass median aerodynamic diameter
P – Pulmonary region of the respiratory tract
PCR – Polymerase chain reaction
PD TESS – Product Director, Test Equipment, Strategy and Support
PFU – Plaque-forming units
PM10 – Particulate matter with a diameter less than or equal to 10 μm
RNA – Ribonucleic acid
SARS – Severe acute respiratory syndrome
T&E – Test and evaluation
TB – Tracheobronchial region of the respiratory tract
TRL – Technology readiness level
USAMRIID – United States Army Medical Research Institute for Infectious Diseases
WSLAT – Whole system live agent testing