UCRL-JC-135368
PREPRINT

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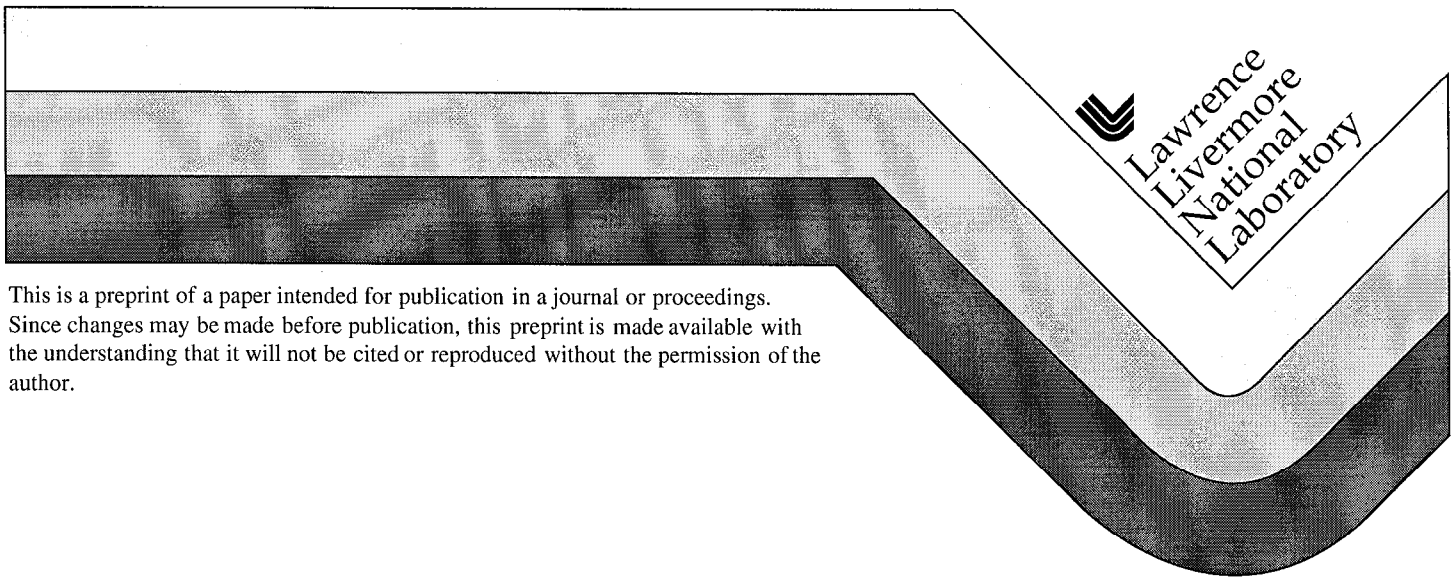
This paper was prepared for submittal to the
24th Session of the International Seminars on Planetary Emergencies
Erice, Italy
August 19 - 24, 1999

August 1999



PROGESS IN RAPID DETECTION AND INDENTIFICATION OF UNKNOWN HUMAN AND AGRICULTURAL PATHOGENS

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I. INTRODUCTION

The biological revolution is providing new knowledge on individual life forms and their related biochemistry, on how living things interact with each other, and on new means to measure and quantify their biological properties. This knowledge is rapidly changing medicine, agricultural, environmental practice, and public health. The collective effects of this revolution are influencing qualities of human life and behavior in ways never before imagined. However, along with the positive aspects of this revolution, come potential negative aspects (Dando[1], Sidell[2]). They include, to name a few, an increased potential for human plagues caused by increased rates of human contact and resistance to antibiotics (Satcher[3]), agricultural plagues exacerbated by extensive use of single-genetic-strain crops and livestock, and purposefully induced plagues of human or agricultural pathogens (Rogers[4]). Past examples of unfortunate biological events include the late-blight of potatoes which led to the Irish famine of 1845-46 causing up to 1 million deaths, the flu pandemic of 1918 which resulted in 20 million deaths world-wide, and the purposeful use by Japanese forces of human disease organisms in China during WWII (Christopher[5]). More worrisome potential events in the future include the development and delivery of biological weapons for purposes of mass destruction. Examples of these are the Iraqi developments of Scud missile delivery systems in 1991 and the Aum Shinrikyo cult's attempts to use biological weapons in Tokyo in the 1990-1994 time frame (Wilkening[6]).

The contemporary revolution in biochemistry, genetics, biology, medicine and related topics is leading to an understanding of how biological agents operate in varied environments and to increasingly sophisticated methods of agent detection. Much of the international medical and agricultural industries' research is directed to the detection and identification of pathogens and to understanding their biological behavior. A consequence of this knowledge is the availability of increasingly effective vaccines, curative agents, and detection systems. In particular, these efforts are leading to pathogen detection systems that are lower in cost, more bio-chemically specific, more accurate, faster, smaller, less demanding of infrastructure, and more accessible to more people than ever before.

The purpose of this paper is to describe recent progress in developing rapidly acting, specific, and low cost detection systems for a very wide variety of pathogens and their related agents. The reasons and procedures for the purposeful uses of

biochemical and chemical agents, by military or terrorists, is discussed more thoroughly elsewhere (Dando[1], Sidell[2]).

II. DETECTION OF BIOLOGICAL PATHOGENS:

The major problem with biological pathogens is that individuals can be infected with relatively few spores, cells, or toxin molecules—as low as a few 1000 agent-entities. Furthermore, initial symptoms of biologically replicating agents are often "flu-like," which are not indicative of the internal problem of an infected host. By the time specific external symptoms appear, it is usually too late. Since most humans or animals can easily contact 1000 infecting agent-entities within a few10's of minutes of breathing or by nominal fluid ingestion, there is a premium on rapid detection. In addition, there is a need for available protective measures to prevent further ingestion (if detected) and a need for appropriate drugs or vaccines to prevent action of the agent in the host. Early detection of agent presence and rapid screening of potentially infected populations is of high value.

Two major techniques for meeting these needs are discussed. The first is the automated, rapid analysis of the nucleotide sequences of suspected agents (e.g., DNA, or transcribed RNA) and the second is the fluorescent detection of labeled antibodies that specifically attach themselves to characteristic proteins (or other chemicals) associated with pathogen chemistry. Many detection and identification techniques have been developed over the years, and are practiced worldwide today. They include host disease-symptom analysis, surrogate animal or plant infection and analysis, antibody tests, growth in cultures, visual agent identification using microscopes of varying types, spectroscopic analysis of chemical or atomic characteristics, etc. These past methods suffer from the slowness of the processes, a lack of specificity, excessively high error rates in the chemistry, a need for relatively large samples, high reagent costs due to relatively large use per test, and extensive technician handling with consequent errors. Progress in solving these problems is being made through advances in biochemistry (especially in fluorescence labeling) and efficient integration of the chemistry and related process steps, using modern micro-electronic, -mechanical, -fluidic, and micro-optical technologies.

III. NEW DETECTION APPROACHES:

Two general sampling approaches are being used to obtain samples for practical high-speed analysis. The first uses procedures to sample the environment, usually air or water (i.e., fluids), for the presence of dangerous organisms. These require sampling up to 1000 liters of air or many liters of fluid each minute, for extended periods of time, to obtain enough pathogen entities. The second procedure is for rapidly processing large numbers of different samples that have higher concentrations of pathogens. Fluids or tissues from infected hosts or concentrated samples from the large flow rate systems lead typically to milliliter sized samples. The system constraints are usually associated with efficiently processing large numbers of samples of soil, plant or animal materials, or many human fluid samples (e.g., 100's to 1,000,000's at milliliters each). Large volume flows and large numbers of samples contain very heterogeneous background organic and inorganic chemicals and materials that complicate the search for the relatively low number of targeted pathogen entities. Fig. 1 below shows a system for pathogen collection, processing, and detection from air samples.

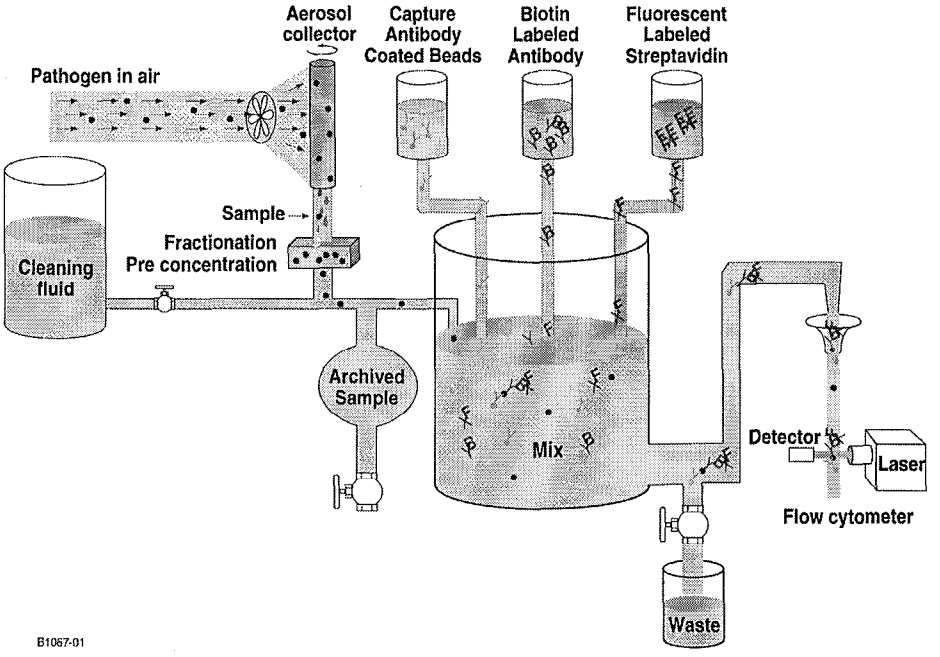

Figure 1. A process diagram for pathogen concentration, fluidization, pathogen labeling using antibody derivatized polystyrene beads, biotin labeled antibodies which then attach to the bead-protein complex, fluorophore labeled streptavidin for labeling of the biotin activated complexes, and flow detection (i.e., flow cytometry).

Reliable procedures are being developed for sample acquisition, sample labeling, background removal or suppression, and pathogen preparation (i.e., concentration, labeling, etc.). Once the pathogen is prepared, it can be reacted with one or more appropriate reagents, amplified (if nucleotide based), flowed through an identification system for statistically meaningful signal detection, it can be correlated with other data, reports prepared, results communicated and archived. Sampling tasks range from a few 100s of exposures to be identified in minutes (in small-unit military situations), to 10,000's of potential exposures in confined environments to be analyzed in day(s) (e.g., people in stadiums), to several millions of samples over month time periods, (e.g., blood bank quality control).

One of the most useful technologies is nucleotide detection (e.g., DNA, or transcribed RNA). Useful references are Kwok[9] and Burger[10]. The nucleotide marker for replicating pathogens is especially useful because it is a long chain molecule (either whole or in pieces), that contains an enormous amount of information on the agent. This information can be used for pathogen detection, pathogen species, source of pathogen, mutation history, processing information, etc. In addition detection techniques based upon nucleotides are able to make use of the PCR reaction techniques (see Mullis[11, 12], Saiki[13]). These enable the use of very small samples of a suspected pathogen (e.g., 10-100 cell or spore entities in a sample) to be rapidly amplified (i.e., multiplied) to very large numbers for subsequent analysis. The analysis is based upon a reacting fluorescing reagent using well-known (and constantly improving) substrate and fluorescence molecule combinations for sensitive optical detection. Because it is difficult to detect single fluorescing antibody antigen complexes, methods are being devised to concentrate the antigens so the fluorescing species density and number is sufficient for reliable detection. Methods to do this include "DNA chips" (Cheng[14], Christel[15]) and antibody derivitized spheres that attract multiple antigens to the sphere surface.

Protein (or other) chemical characteristics of pathogens (i.e., antigens), including specific membrane or spore coating chemistries, and associated carrier chemicals are also attractive targets for antibody attachment and associated fluorescence marker detection. These antigens can not be amplified, thus for these techniques to work well a sufficiently large quantity of pathogen must be obtained. They can then be concentrated so that efficient detection can take place. By introducing antibody derivatized detection systems (e.g., polystyrene spheres with antibody coatings) into liter-sized fluid samples, and allowing sufficient time for thorough mixing and antibody/sphere diffusion (e.g., 10 min.), complete "sampling" all molecular entities will occur. This will ultimately result in binding to targeted pathogenic chemicals or antigens. Then a fluorescing antibody can be attached to the antigen-sphere complex, and the host fluid can then be flowed through a liquid jet where they are detected (Lindmo[16]). In the channel, the complexes are caused to fluoresce by single or multi-color laser illumination. The detected entities are concentrated by removal from the host-fluid stream using jet deflection techniques (Langlois[17]). Today, using such techniques, multiple antibodies with multiple fluorescing chromophores are being sampled in small, compact, high-speed fluid sorters.

IV. MINITURIZATION OF DETECTION SYSTEMS:

The issues of detection involve acquisition of purported pathogen samples and separation from the background environment (e.g., air, dust, pollen, other bacteria and fungi in the air, etc.), determining a detection specific set of reagents, and developing a sufficiently rapid rate of sample acquisition, processing, and data processing. The techniques for filtration and sample concentration, for sample preparation, and for data analysis, reporting and record keeping will not be described further here as they, in principle, can be engineered to be consistent with the pathogen technologies described below.

Small sample volume polymerase chain reaction (PCR) detection systems have advantages for detection (Hsueh[18], Belgrader[19]) over more traditional approaches. These advantages include low costs of reagents, attractive physics of heat flow for rapid temperature cycling, improved signal to noise for low pathogen "count" detection, and rapid and controlled transport of fluid samples. Fig. 2 below shows a chip-based, thin chamber system can control the thermal cycle that is necessary for the PCR to work accurately and rapidly. (Tens of seconds).

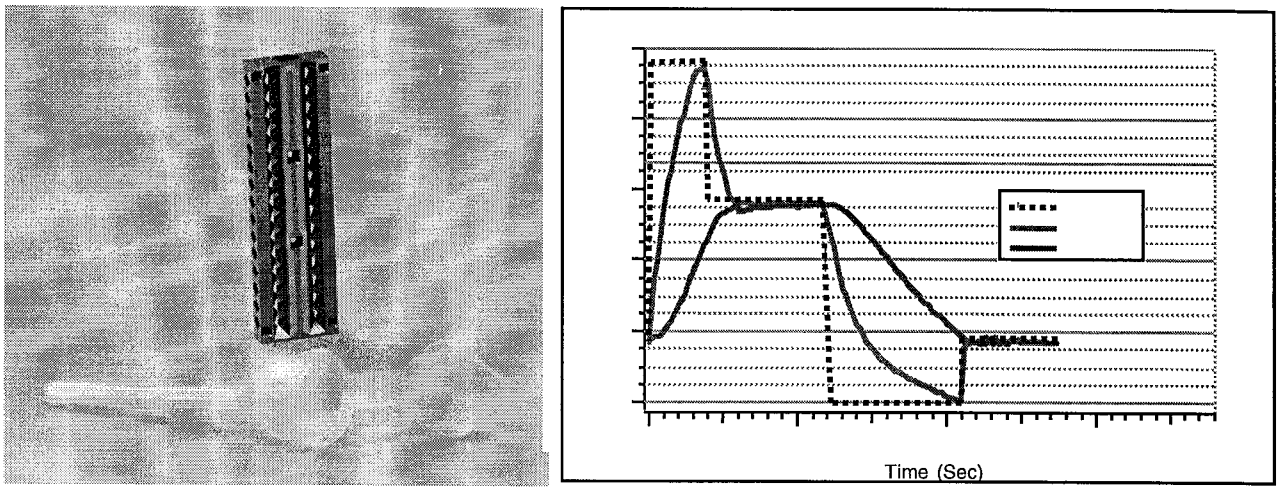

Figure 2. Silicon thermal cycler used for rapid polymerase chain reaction detection and corresponding thermal profiles for heater and internal sample. (Belgrader[20])

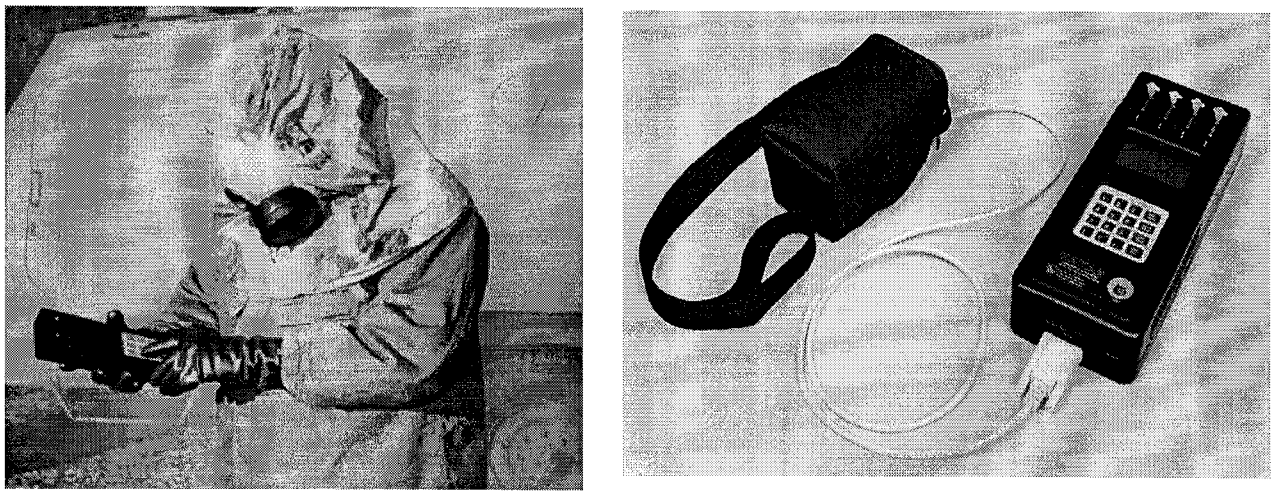

Figure 3a and 3b. Miniaturized biochemical detection systems are making possible low cost rapid, portable pathogen identification. (Hurt[21], Ibrahim[22])

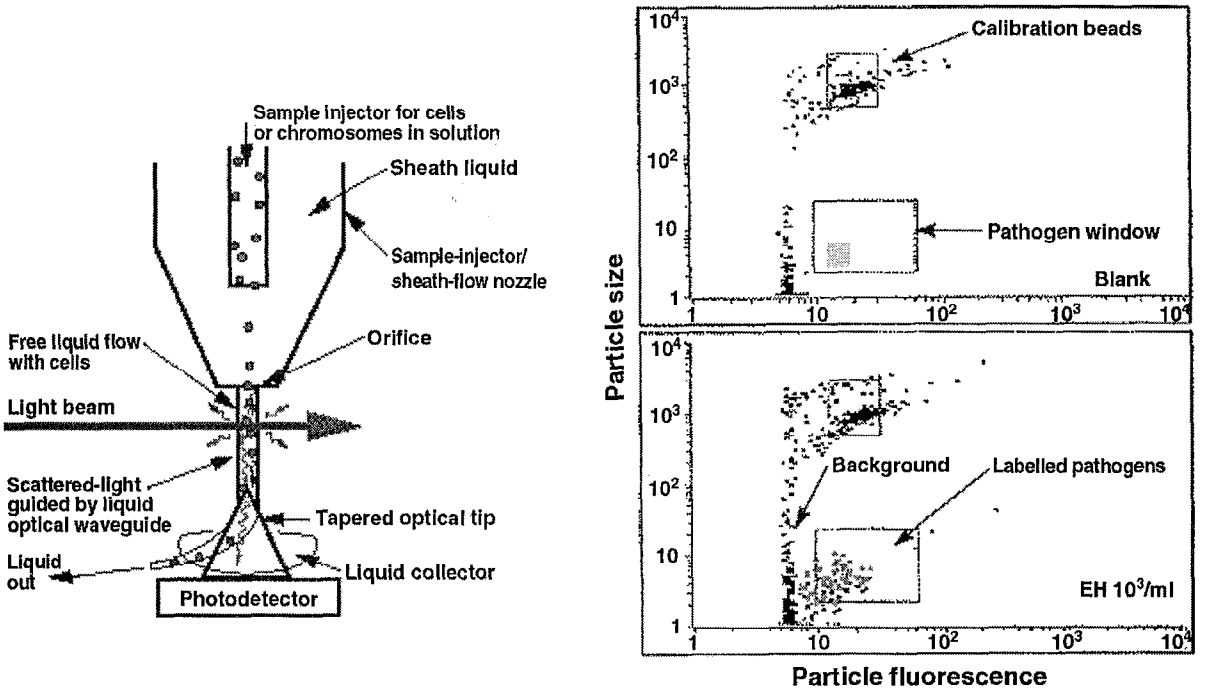

Figure 4. (a) Schematic for a miniaturized flow detection system for detecting pathogens labeled with fluorescent antibodies. (b) Data from the miniaturized flow cytometer-showing detection of a bacteria (*E. herbicola*) at a concentration of 1000 bacteria/ml. (Mariella[23])

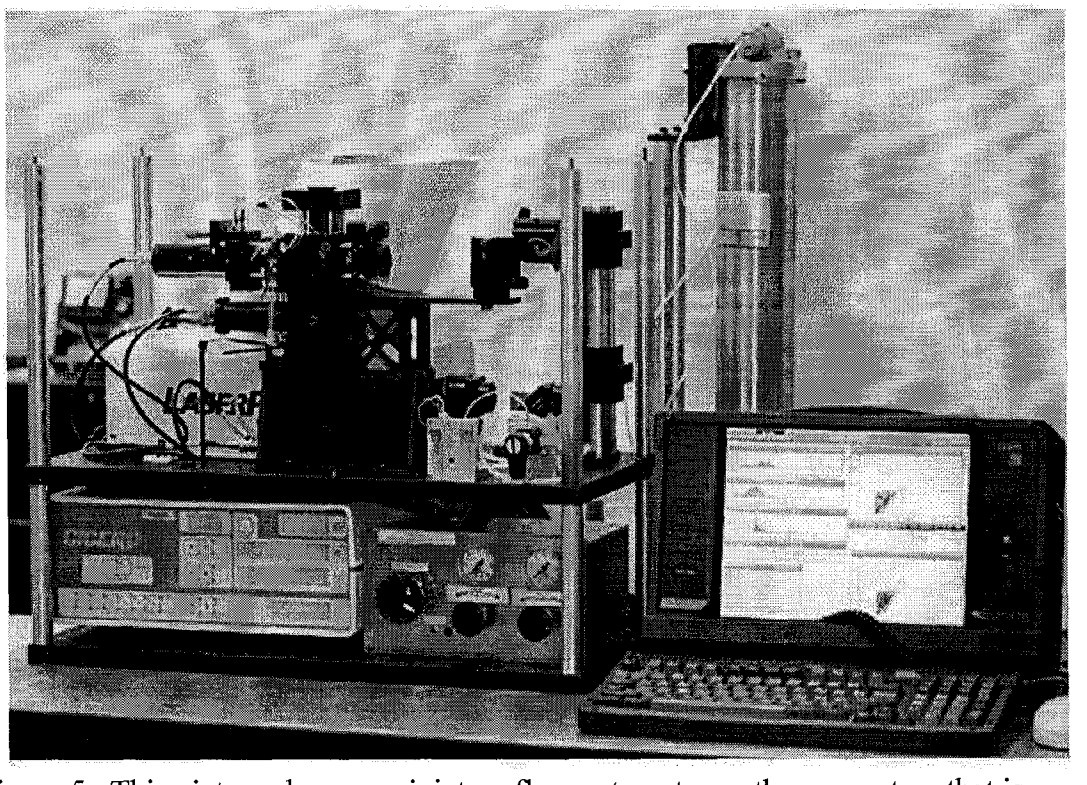

Figure 5. This picture shows a miniature flow cytometer, pathogen system that is becoming ready for miniaturization. This system has performed well in field studies, exhibiting sensitivities to below 10^3 cells on spores/ml. (Mariella ibid)

V. CONCLUSION:

The medical industry is driving pathogen detection technology from its present characteristics of $50/sample, 100 sample capability systems, with several day time responses, having several percent error rates in reported outcomes. The systems described above are capable of providing samples at < $5/test, managing several million samples, < 1-hour cycle times, (or just minutes in some cases) and < 0.1% error rates. Because of their importance to the medical and agricultural communities, all "important" pathogens will have detection kits available (within air transport times, anywhere in the world) by 2020, and the most well known pathogens will have kits available within a few years. Many are available now.

Because of the importance of the food supply to modern nations, these technologies will be employed everywhere in this industry. For example, the United States imports 30 B tons of food a year, but inspects < 1%. Portable inspection systems will make it possible to test for dangerous pathogens in feed lots, food processing plants, markets, and points of use. Outbreaks of animal or plant disease will be immediately detectable using field instrumentation, and more complex samples can be sent to central testing laboratories where more sophisticated test systems will be available.

Unusual pathogens either naturally or purposefully selected or developed, will require special attention because there is not a commercial economic driver for the development of detection systems and curative agents. Their development, and production for sufficient availability, will require significant investments by the world community. The strategy and costs for developing vaccines or curative drugs will be very expensive and will need special attention. However it is important that attention be directed to these problems because such attention has a strong deterrent effect on potential developers or users. The capacity to use the full information content contained in pathogen systems, such as their full genomic information, can be very helpful in identifying malevolent users. In addition, it is undoubtedly true that an understanding of replication and human or other sensitivity to pathogens will improve our medical understanding of human health in general.

ACKNOWLEDGEMENTS:

We would like to thank Alan Northrup, Ph.D. of Cepheid Inc., and Dean A. Wilkening, Ph.D. Center for International Security and Cooperation, Stanford University. I would also like to thank Ray Mariella, Jr., Ph.D. and Rod Balhorn, Ph.D of Lawrence Livermore National Laboratory.

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This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.