FINAL REPORT

DOE award number: **DE-F C52-04NA25455**

Name of recipient: **National Center for Biodefense and Infectious Diseases, George Mason University.**

Project title: **Rapid Detection of Biological and Chemical Threat Agents Using Physical Chemistry, Active Detection, and Computational Analysis**

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11/2005 through 5/2012

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Executive summary.

Provide an executive summary, which includes a discussion of 1) how the research adds to the understanding of the area investigated; 2) the technical effectiveness and economic feasibility of the methods or techniques investigated or demonstrated;

or 3) how the project is otherwise of benefit to the public. The discussion should be a minimum of one paragraph and written in terms understandable by an educated layman.

Basic technologies have been successfully developed within this project: rapid collection of aerosols and a rapid ultra-sensitive immunoassay technique. Water-soluble, humidity-resistant polyacrylamide nano-filters were shown to (1) capture aerosol particles as small as 20 nm, (2) work in humid air and (3) completely liberate their captured particles in an aqueous solution compatible with the immunoassay technique. The immunoassay technology developed within this project combines electrophoretic capture with magnetic bead detection. It allows detection of as few as 150-600 analyte molecules or viruses in only three minutes, something no other known method can duplicate. The technology can be used in a variety of applications where speed of analysis and/or extremely low detection limits are of great importance: in rapid analysis of donor blood for hepatitis, HIV and other blood-borne infections in emergency blood transfusions, in trace analysis of pollutants, or in search of biomarkers in biological fluids. Combined in a single device, the water-soluble filter and ultra-sensitive immunoassay technique may solve the problem of early "warning type" detection of aerosolized pathogens. These two technologies are protected with five patent applications and are ready for commercialization.

Comparison of the actual accomplishments with the goals and objectives of the project.

The two major goals of the project were (i) development of a rapid ultra-sensitive immunoassay technique and (ii) development of a rapid and economic method of aerosol collection compatible with the immunoassay method. Combination of these two methods provides a basis for design of a "warning type" sensor for aerosolized pathogens. In accordance with these major goals the following tasks were performed:

Task 1. Development of rapid ultra-sensitive detection techniques for biological threat agents.

Integration of an electrophoretic cell for active capture of pathogens onto an array of antibody molecules with a flow cell for active scanning of the array with functionalized magnetic beads in search of bound pathogens. This challenge requires solving engineering problems such as connection of the device to the electrode chamber without interfering with illumination and optical imaging, design of membranes with adequate mechanical stability, allowing access to magnets, permitting effective heat exchange, and others. We plan to design and test several prototypes in search of an optimal design.

The task was successfully completed by designing and testing seven prototypes of the electrophoretic cell, flow cell and combined cells until a final prototype was developed which solved all the technical problems encountered. The design is protected by two patents (pending applications). A theoretical analysis of electrophoretic collection within the flow cell was performed to identify conditions combining the highest assay speed with the lowest detection limit.

Task 2. Development of rapid ultra-sensitive detection techniques for biological threat agents.

We plan to further decrease the limit-of-detection (LOD) of active detection by using newly developed membranes with a surface that is flat at the nano-scale. Antibody immobilization onto beads and onto the array will also be modified to obtain maximal sensitivity to toxins and pathogens.

A microarray of antibodies on a semi-permeable membrane is a key component of the electrophoretic flow cell. As a result of studies completed during the project, a combination of membrane properties (mechanical, electrical, optical) has been found which provides the optimal assay performance. In particular, a special “lacquering” procedure has been developed to modify the surface properties of dialysis membranes to make them flat at a nano-meter scale and to enable covalent links with immobilized antibody molecules. The stability of immobilized probe protein molecules after storage

and their performance in scanning detection were characterized. The task was successfully completed by lowering the limit of detection in a 3 minute immunoassay to an incredible zeptamolar level, corresponding to only 150-600 molecules in a probe volume of 0.1 mL. This level of sensitivity has previously been achieved only in PCR-based assays and in a highly complex immunoassay procedure requiring hours to complete.

Task 3. Development of rapid ultra-sensitive detection techniques for biological threat agents. *It has been demonstrated in our experiments that capture of microbial pathogens, like B. anthracis, on an array of specific antibodies can be quickly and effectively performed in a centrifuge. We planned to study centrifugal capturing of pathogen cells and spores.*

After successful development of the ultra-sensitive detection technique described within Task 1, the latter was applied to detection of viruses found in the blood of chicken infected with West Nile virus. It was demonstrated that 500-700 viruses can be detected in 2-3 minutes using our detection technique. Thus, ~1000-fold fewer viral particles can be detected in ~100-fold less time using our technique than in conventional immunoassay methods. The task has been completed successfully.

Task 4. Development of rapid ultra-sensitive detection techniques for biological threat agents.
Develop a mathematical algorithm and software for analysis of bead binding.

It was found during the project that magnetic beads bind strongly enough to the antibody spots and accumulate at sufficient levels to allow the use of still digital images as an analysis tool. In view of this, no special mathematical algorithm was found to be necessary. It was also determined that image analysis software developed by NIH and freely available as the Windows version of Scion Image can be successfully used for analysis of bead binding. Thus, the task was fulfilled using available techniques.

Task 5. Development of aerosol technology.

Design of a nano-aerosol generator based on the collision of oppositely charged electrospray-generated products.

This task was successfully completed by designing a nano-aerosol generator which employs a new principle. It was demonstrated that aerosol particles of different size and shape can be produced with this new technique by changing the solution composition or the electrospray conditions, such as air humidity.

Task 6. Development of aerosol technology.

Test the functional activity of enzymes and antibody molecules as well as the viability of cells and viruses in the nano-aerosols manufactured by the new technology.

Using alkaline phosphatase as a model system, we detected no notable decrease in enzyme activity upon fabrication of nano-aerosols using a new electrospray-neutralization technology developed within this project. The viability of cells and viruses has not been tested due to the lack of a safe containment chamber.

Task 7. Development of aerosol technology.

Use the nano-aerosol generator to fabricate nanofilters for retention of aerosol particles.

The task has been successfully completed. A variety of highly effective nano-filters was prepared using our novel electrospinning-neutralization technique (patented). It was demonstrated that these filters have a far superior combination of high aerosol capture and low resistance to air flow than conventional fibrous filters. Our filters are appropriate for a variety of applications including nasal filters, technical filters for air, water and oil, or filters for analysis of air pollutants like pollen and pathogens. In consideration of the major goal of the present project, rapid economic collection of aerosolized pathogens, we have developed water-soluble, humidity-resistant filters that can collect aerosol particles in a humid atmosphere and then completely liberate the

collected particles into a small volume of an aqueous solution compatible with immunoassay techniques. It was shown that these filters are able to collect nano-aerosol particles as small as 20-50 nm and withstand pressures up to 0.5 atm in humid air. Considering their ability to liberate all bound particles and adsorbed toxins upon dissolution, these filters provide a practical alternative to standard aerosol collectors, such as impactors and cyclones, which require significant power and are ineffective in collection of sub-micron aerosol particles.

Summarize project activities for the entire period of funding, including original hypotheses, approaches used, problems encountered and departure from planned methodology, and an assessment of their impact on the project results. Include, if applicable, facts, figures, analyses, and assumptions used during the life of the project to support the conclusions.

In our previous DOE funded project "Rapid Detection of Biological and Chemical Threat Agents Using Physical Chemistry, Active Detection, and Computational Analysis" (**DE-F C52-04NA25455,** 6/2004 through 10/2005) we evaluated methods of rapid economic collection of aerosol particles and methods of rapid analysis of collected samples for the presence of pathogens (toxins, viruses, spores). We arrived at the conclusion that combination of the active immunoassay with collection of aerosolized pathogens on a water soluble filter would solve the problem of rapid "warning type" early detection of aerosolized threats. All our efforts during the 18-month period of this project were focused on solving numerous technological problems in this direction.

One major goal has been achieved by design of a cell which allows both electrophoretic capture of pathogens from flow and detection of the captured pathogens with magnetic beads using a scanning detection technique. Numerous technical problems have been solved in series of prototypes designed and tested: effective cooling of the analyte solution, transparency of the electrode chambers, prevention of clogging of magnetic beads in the magnetic field, avoiding interference from bubbles generated on electrodes, and many others. A refinement of the cell design, improvements in the technology of immobilization of arrayed antibody molecules, and a theoretical analysis of the capturing and detection processes resulted in a dramatic ~1,000 fold decrease in the

LOD and ~10 fold decrease in assay time as compared to the state of the active assay technology at the beginning of the project. At present, our active assay technique is the most sensitive and the most rapid immunoassay known. It is important to emphasize that the technique is capable of detecting pathogens in highly crowded samples: it was shown that a 10^{11} excess of other proteins in the serum sample does not prevent specific antigens from being detected. We envisage many potential applications of this technique in medicine, science, and the monitoring of environment pollution.

Rapid energy-efficient collection of aerosol particles with micron and sub-micron sizes was a second major challenge of the present project. The following problems have been solved in the development of water-soluble, humidity-resistant filters: (i) synthesis of a special polymer material; (ii) characterization of its spinnability in terms of polymer viscosity and entanglement of polymer chains; (iii) characterization of the polymer's ability to form reversible cross-links (mechanical and swelling properties); (iv) characterization of filter performance in terms of capturing efficiency to particles of different sizes, resistance to air flow, air pressure; (v) characterization of humidity effects on filter storage and operation; and (vi) choice of conditions providing rapid dissolution of the filter. All these studies were conducted using a polyacrylamide-based polymer with introduced side-chain SH-groups, and a prototype of a water-soluble, humidity-resistant nanofilter has been created which withstands a day-long storage at 100% humidity and 10-15 minutes collection of particles from air with 95% humidity without notable changes in collection efficiency or air resistance. The filter dissolves within 1-2 minutes in a reducing DTT solution compatible with antibody-based assay techniques. The filter is capable of collecting 100% of particles with a diameter 1 μm, 80% of particles with a diameter of 100 nm and 60% of particles with a diameter of 30 nm, and consumes much lower energy as compared to cyclones or impactors. Therefore, the filter meets the challenge of the project. It can be used both in combination with the active assay device described above and in other applications where economic, rapid and highly effective collection of very small particles, like separate viruses, is required. In particular, the extremely high sensitivity of the active assay technique in combination with highly efficient collection of aerosols may be used to analyze airborne infections in hospitals, airports and other public buildings, or to make diagnostics by analysis of patient breath.

In conjunction with the present project, a related project was performed which was aimed at development of a rapid diagnostic assay for the early detection of West Nile virus (WNV) infection (in collaboration with USAMRIID). It was demonstrated that the active assay technique is capable of rapid detection of a small number of West Nile viruses (500-600 WNV in 3 minutes) in serum samples from chickens that have been infected with WNV. It was also shown that antibody molecules specific to WNV could be discovered in chicken serum very rapidly (15 minutes against 2 days in the standard commercial immunoassay kits).

Products developed under the award and technology transfer activities:

a. ***Publications in peer-reviewed journals:***

1. Morozov**,** V.N., Morozova, T.Ya. Recognition of Closely Related Antigens by Active Immunoassay. (2007) *Anal. Biochem., in press.*
2. Groves, S., Turell, M. J., Bailey, C., Morozov, V.N. (2007) Rapid active assay of pathogen-specific antibodies in chickens infected with West Nile Virus. *Am. J. Trop. Med. Hyg. , in press.*
3. Vetcher, A.A., Gearheart, R., Morozov, V.N. (2007) Electrospun, Humidity-Resistant, Water-Soluble Nanofilters. *Polymers for Advanced Technologies. in prep.*
4. Morozov, V.N., Vsevolodov N.N. (2007) Electrospray-neutralization method for manufacturing free and supported nanomats. *Adv. Materials, in press.*
5. Morozov, V.N., Groves, S., Turell, M. J., Bailey, C., (2007) Three minutes-long electrophoretically assisted zeptomolar microfluidic immunoassay with magnetic beads detection. *J. Am. Chem. Soc.,* **129**, 12628 -12629.
6. Vetcher, A.A., Gearheart, R., Morozov, V.N. (2007) Correlation of morphology of electrospun fibers with rheology of linear polyacrylamide solution. *Polymer J.,* **39,** 878-881.

Copies of publications are attached.

b. Web site or other Internet sites that reflect the results of this project;

1. http://math.gmu.edu/faculty_staff/napoletani.htm
2. http://ncbid.gmu.edu/faculty.cfm?id=35&menu_id=2
3. http://ncbid.gmu.edu/page2.cfm?menu_id=14&sub_menu_id=0

c. Networks or collaborations fostered;

1. Cooperative Research and Development Agreement U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

e. Inventions/Patent Applications:

1. Morozov, V., Vsevolodov, N., A. Elliott, A. Bailey, C. (2006) Detection and recognition of analytes. USPTO Application # **20060275824, December 7, 2006**
2. Morozov, V., Evanskey, M., Bailey, C. (2006) Analyte detection using an active assay. USPTO # **20060263904, November 23, 2006**
3. Morozov, V. (2006) Flow chamber , USPTO #**20060263269, November 23, 2006,**
4. Morozov V. (2006) Electrocapturing flow cell. USPTO #**20060260942, November 23, 2006**
5. Morozov, V., Bailey, C. (2006) Electrospray Neutralization Process and Apparatus for Generation of Nano-Aerosol and Nano-Structured Materials. USPTO**, # 20070113530, May 24, 2007.**

d. instruments or equipment.

The following prototypes of devices and products have been designed and tested:

1. Prototype of a device for rapid ultra-sensitive detection of toxins, antigens, antibodies and viruses including (i) patented flow cell with electrophoretic collection of analytes onto an array of specific probe molecules (e.g., antibodies), optical microscope with dark-field illumination, CCD camera, two syringe pumps, power supply and computer. Flow cell design is patented.
2. Prototype of a generator for manufacturing nano-aerosols (patent is pending).
3. Prototype of a device for manufacturing nano-filters and free nanomats based on a new principle (patent is pending).
4. Prototype of a nasal filter.
5. Prototype of a water-soluble, humidity-resistant filter for rapid collection of aerosolized pathogens for immunoassay detection (patent is pending).