Information Paper

Production of gamma-irradiation sterilized dried *Bacillus anthracis* spores.

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The following information is provided as per request:

1. All documents relating to dry or powdered *Bacillus anthracis* to include:

   a. The nature and scope of each project (including identification of classified projects)
   The only project that we have been involved with concerning dried *B. anthracis* spores was part of a DARPA project as funded through (b) (6) (DARPA). As part of our Memorandum of Agreement, two DARPA contract hires (b) (6) and (b) (b) (6) along with the undersigned and his technician, (b) (6) produce purified *B. anthracis* spores for analysis using mass spectrometry. Spores from both the Ames strain and the Sterne strain were grown, purified using Renocal 76 density gradients, enumerated, sterilized via gamma-irradiation, sterility checked and freeze dried (lyophilized) in sealed vials. No dried live spores were prepared.

   b. The following protocol was used (verbatim):

   Preparation of spores: *Bacillus anthracis* Ames and Sterne strains were aerobically grown overnight on (b) (4). These starter cultures were used to make a medium to heavy suspension of cells in (b) (4). Approximately (b) (b) of this suspension was spread onto (b) (4) culture plates (b) (b) (4) in triplicate. Cultures were incubated for (b) (4) hours at ambient temperature. Upon inspection, using phase 4 microscopy, spores were harvested when approximately 90-99% of culture appeared to be refractive spores, via scraping with glass rod spreaders and washing with sterile distilled water. Spores were washed (b) (4) (b) with sterile water and then resuspended in (b) (4) purification procedure. Attempted to purify spores by separation via the density gradient (b) (4) spore suspension was layered on top (b) (4) in (b) (4) centrifuge bottle. Bottles were then placed in (b) (4) and spores separated from vegetative cells via centrifugation at (b) (4) Spores at bottom of bottle were collected and combined, washed (b) (4) with sterile water, then split into halves with one half being (b) (4) and the other half held in (b) (4) An aliquot from each sample was taken in order to quantify the spores. Counts were made of the water/spore suspension by (b) (4) and then dispensed into (b) (4) in (b) (4) screw cap tubes. (b) (4) aliquots of spores in water were then inactivated via gamma irradiation with (b) (4) total exposure. Sterility was performed by plating (b) (4) of irradiated sample in triplicate onto (b) (4) and incubating for (b) (4)
   Preparation of lyophilization vials : To Sterne vials was delivered (b) of spore suspension (b) (4) and to Ames vials was added (b) (4) of suspension (b) (4) ; each set when reconstituted with (b) (4) of sterile water would equal (b) (4) Suspension was frozen in vials and placed on lyophilizer. Post dehydration, vials were sealed, labeled, and crimped. Dried spore weight is not available for this lot of spores.
   Final product lot numbers:
   Bacillus anthracis Ames spores = 30 Jul 97 BASP
   Bacillus anthracis Sterne spores = 30 Jul 97 BSSP

Agar medium used for spore production:

NSM agar per liter

Component	Amount/ liter
Tryptone	(b) (4) [redacted]
Yeast Extract	
Bacto-agar (Difco, Detroit, Michigan)	
Lab-Lemco Agar	
$MnCl_2.4H_2O$ (1% solution)	

Dissolve in 900 ml distilled water

Adjust pH to (b) (4) [redacted], autoclave, (b) (4) [redacted] and pour into sterile Petri plates.

c. Production documents relating to each of dry *B. anthracis* produced, including detail accounting of amount created, strain identification, the disposition of *B. anthracis* produced upon completion of each project. The procedure above is all the has been found to date. All the *B. anthracis* dried spores were destroyed over a year ago due to lack of quality assurance documentation. Two vials were retained, one of which was used to compare to the spores from the Senator Daschle and Senator Leahy letters and the second has remained unopened, at the request of the FBI, for future analysis.

d. A complete list of all persons who have been involved in the production of dry, powdered or dry simulants of *Bacillus anthracis* (i.e. other *Bacillus* species).

i. (b) (6) [redacted]
ii. (b) (6) [redacted]
iii. (b) (6) [redacted] (past employee)
iv. (b) (6) [redacted] (past employee)

All dried materials were sterilized by gamma-radiation prior to drying.

22 January 2002

MEMORANDUM TO: (b) (6) [redacted]

TO: Commander, USAMRIID

SUBJECT: Inactivated Dried Biological Threat Agents

1. As per request, the following information is supplied concerning lyophilized materials that were produced in accordance with a Memorandum of Agreement with DARPA established in FY 97.
2. Two vials of gamma-irradiated killed *Bacillus anthracis* Ames strain spores was remaining from less than 100 vials produced in 1997 by (b) (6) [redacted] for use in meeting DARPA requirements (See included MOA). One of the two remaining vials was provided by the undersigned to (b) (6) [redacted] for comparison with other spore materials using electron microscopy.
3. Other inactivated dried materials include:

Bacillus anthracis (Ames) spores 30 Jul 97
3.26×10^8/ vial QTY - 1 vial

Bacillus anthracis (Ames) vegetative cells
1.8×10^7/ vial QTY - 98 vials

Bacillus anthracis vegetative cells
3.5×10^7/ vial QTY - 144 vials

Brucella abortus ATCC: 4316
Lot: 2Dec97BrA
1×10^8 / vial QTY - 98 vials

Bacillus anthracis Sterne strain spores
Lot: 30Jul97BSSP
5×10^8/ vial QTY - 13 vials

Bacillus anthracis Delta Ames spores
Lot: 02Dec97BADSP
1×10^7/ vial QTY – 79 vials

Yersinia pestis (JAVA9)
2.5×10^7/ vial QTY - 71 vials

Francisella tularensis LVS
Lot: 090297
9/29/98
1×10^7/ vial QTY - 24 vials

(b) (6) [redacted] Ph.D.
C, Special Pathogens Branch
USAMRIID

MEMORANDUM OF AGREEMENT BETWEEN THE JOHNS HOPKINS UNIVERSITY APPLIED PHYSICS LABORATORY AND THE DIAGNOSTICS SYSTEMS DIVISION OF THE U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

SUBJECT: USAMRIID Interaction with DARPA Funded Projects at the Johns Hopkins University Applied Physics Laboratory

I. Purpose: The Johns Hopkins University Applied Physics Laboratory (hereafter JHU/APL) and the Diagnostics Systems Division of the U.S. Army Medical Research Institute of Infectious Diseases (hereafter DSD-USAMRIID) hereby enter into an agreement to cooperate on the development and execution of new technology that is supportive of militarily relevant biological threat agent detection and identification for military and counter-terrorism surveillance measures, thereby sustaining the operational capabilities of U.S. military personnel. The intent of this Memorandum of Agreement (MOA) is to establish and coordinate collaboration between Defense Advanced Research Projects Agency (DARPA) funded research projects at JHU/APL, their subcontractors, and DSD-USAMRIID for the development of new technologies to detect and identify multiple biological threat agents.

II. Background: A biological warfare agent detection program, funded by the DARPA is underway at JHU/APL to develop miniaturized time of flight (TOF) mass spectrometers to perform analyses in real time. JHU/APL and their subcontractors bring to this effort a high level of expertise in mass spectrometry. DSD-USAMRIID, with its expertise in biological threat agents, will enhance the efforts at JHU/APL to develop technologies for rapid identification of biowarfare agents in the battlefield. Although not part of this MOA, the miniaturized TOF mass spectrometers promise to have application as a tool for diagnosis in the medical arena.

III. Subject to DARPA funding and direction, the Parties shall:

a. Cooperate on research projects aimed at developing technologies for detection and identification of biological threat agents until one of the following events occurs: DARPA funding has ended, the objectives are achieved, or it is deemed by either party that no further collaboration is warranted, whichever occurs first.

b. Keep the other parties fully informed of progress on the joint development throughout the length of the agreement. A productive collaboration shall be fostered through communications to review data, exchange information pertinent to the studies and plan future experiments. Quarterly or as needed scientific and management reviews shall be conducted with all parties participating.

c. Disclose collaborations with outside groups on matters relating to project. Develop a joint annual investigational plan for work to be completed at DSD-USAMRIID, APL, and collaborating labs. The plan will be used for planning and management to determine work load and funding requirements.

d. Collaboratively evaluate the use, application, and future development of mass spectrometry for biological threat agent identification, which would provide means for immediate, accurate identification of militarily important aerosolized pathogens on-site or at the first level of medical care (not covered in this funding - future amendment).

e. Specifically acknowledge the contributions of all participating institutions in publications, reports and briefings resulting from the program.

f. File a joint annual report of results to DARPA including a list of subject inventions, publications and pertinent findings relevant to the development of the mass spectral approach to biodetection of threat pathogens.

IV. JHU/APL will, contingent on DARPA funding:

a. Provide mass spectrometer instrumentation for placement in Biosafety Level-3 containment space provided by DSD-USAMRIID. Provide guidance on, maintenance and repair of mass spectrometer to DSD-USAMRIID personnel.

b. Develop data reduction routines and signal processing techniques to aid in the database building and hierarchical decision structure for mass spectral biodetection.

c. Coordinate the development of analytical approaches to biomarker development with their subcontractors and DSD-USAMRIID.

d. Provide investigators, trained in mass spectrometry, to carry out a joint experimental program with DSD-USAMRIID.

These investigators will comply with all USAMRIID regulations as apply to Institute operations, security, and procedures. They will participate in the Special Immunizations Program as it pertains to their collaborative studies with DSD investigators.

e. Provide training to DSD-USAMRIID personnel in operation of and sample preparation for mass spectrometry.

V. DSD-USAMRIID will as its DOD mission obligation permits:

a. Provide microbiological expertise and consultation regarding methods appropriate on preparations of recognized biological threat agents and other biological materials deemed appropriate.

b. Based on the investigational work and collaborative efforts initiated by JHU/APL and approved by USAMRIID, provide biological materials such as inactivated bacterial cells or spores, viral particles or inactivated toxin preparations of threat agents. Active biological materials that can be safely handled at the BL-1 and BL-2 biosafety levels will be supplied to JHU/APL and its subcontractors as required.

c. Based on the investigational work plan initiated by JHU/APL and approved by USAMRIID, provide facilities to accommodate a bench top mass spectrometer within a Biosafety Level-3 (BL-3) containment lab to allow work with active biological threat agents and provide a mechanism for transfer of spectral data to JHU/APL.

d. When required and in accordance with all applicable regulations, will vaccinate, provide apparel and respiratory protection as needed, guide and train APL investigators and staff on the handling of biological agents that require high levels of containment.

e. Provide samples that can be traced to gold standard assays for comparison with the mass spectral technique for identification.

VI. Funding: DSD-USAMRIID and JHU/APL will present an annual joint plan to DARPA for separate but coordinated funding. The level of funding sought will be mutually determined to accomplish the program goals.

VII. Patent Rights:

a. Reporting. The parties shall promptly report to each other all Subject Inventions reported to either party by its employees. All subject inventions made during the performance of this Agreement shall be listed in the Final Report required by this agreement.

b. Employee Inventions. APL shall have the option to retain title in Subject Inventions made by its employees. APL shall notify USAMRIID promptly upon making this election and agrees to timely file patent applications on APL's Subject Invention at its own expense.

c. USAMRIID Employee and Joint Inventions. USAMRIID shall have the initial option to retain title to, and file patent applications on. Each Subject Invention made by its employees. Any invention patentable under US patent law which is conceived or first reduced to practice under his agreement shall be owned by the party entitled to ownership under US patent law. Any investigation arising under this agreement is subject to the retention by the US Government of non-exclusive, irrevocable paid up license to practice or have practiced the invention throughout the work by or on behalf of the US Government.

d. Filing of Patent Applications. The party have the right to retain title to and file patent applications on. A specific Subject Invention may elect not to file patent applications provided its so advises the other party within 90 days from the date it reports the Subject Invention to the other party. Thereafter, the other party may elect to file patent applications on the Subject Invention agrees to assign its ownership interest in the Subject Invention to the other party.

e. Patent Expenses. The expenses attendant to the filing of patent applications shall be borne by the party filing the patent application. Each party shall provide the other party with copies of the patent applications its files and any Subject Invention along with the power to inspect and make copies of all documents are retained in the official patent application files by the applicable patent office. The parties agree to reasonably cooperate with each other in the preparation and filing of patent applications resulting form this Agreement.

VIII. Technical Data and Proprietary Information:

a. Technical Data Ownership. Data generated as a result of this agreement, shall be jointly owned by the parties. Either party shall have the right to review all Subject Data which has not been delivered to the other party.

b. Proprietary Information and Materials. Each party agrees that any Proprietary Information furnished to the other party under this Agreement, or in contemplation of this Agreement, shall be used, reproduced and disclosed only for the purpose of carrying out this Agreement, and shall not be released to third parties unless written consent to such release is obtained from the other party(ies). Materials provided by each party will not be transferred to third parties unless written consent is obtained. Each party will assume that all information pertaining to the joint program is proprietary unless mutually deemed otherwise.

c. The parties agree to confer prior to release the results of research under this Agreement for publication to assure that no Proprietary Information is released and that patent rights are not jeopardized. Prior to submitting a manuscript for review which contains the results of the research under this Agreement, or prior to publication if not such review is made, each party shall be offered an ample opportunity, not to exceed 1 month/30 days, to review any proposed manuscript and to file patent applications in a timely manner.

IX. Offices of Primary Responsibility:

a. U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

1. (b) (6) [redacted]
Deputy Commander
USAMRIID (MCMR-UIZ-A)
1425 Porter Street
Fort Detrick MD 21702-5011
COMM: 301-619-2772
DSN: 343-2772
FAX: 301-619-4625
E-mail: (b) (6) [redacted] @det ick.army.mil

b. Johns Hopkins University Applied Physics Laboratories

1. (b) (6) [redacted]
The Johns Hopkins University
Applied Physics Laboratory
Johns Hopkins Road
Laurel, MD 20723-6099
COMM: 301-953-6210
FAX: 301-953-6904
E-mail: (b) (6) [redacted]@aplcom.jhuapl.edu

2. (b) (6) [redacted]
The Johns Hopkins University
Applied Physics Laboratory
Johns Hopkins Road
Laurel, MD 20723-6099
COMM: 301-953-6000, ext 4392
FAX: 301-953-6904
E-mail: (b) (6) [redacted]@jhuapl.edu

X. Discontinuance Conditions:

a. This agreement may be amended at any time upon mutual consent in writing of the parties concerned.

b. This agreement may be terminated by either party by giving at least 90 days written notice.

XI. Effective date: This agreement shall be effective upon the signature of parties listed below.

(b) (6) [redacted] _____
Colonel, Veterinary Corps
Commander, U.S. Army Medical
Research Institute of
Infectious Diseases

Date: _____

(b) (6) [redacted] _____
The Johns Hopkins University
Applied Physics Lab

Date: _____