

Contaminated Human Remains: Transportable Decontamination Technical Readiness Level Estimate

Project TRL Report

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The purpose of this report is to provide the technical readiness levels for a transportable system for radiation sterilization of human remains. The levels reported are:

Standard TRL 5

Biomedical Device TRL 4

Project TRL 3

A. Background

The inability to safely return biologically contaminated remains to the families of service members who died in defense of their country has been a persistent problem. Many solutions have been explored, including mandatory cremation, but none of them have proven to be both fully technically and politically/socially acceptable. These concerns have been reviewed and documented in “Initial Capabilities Document for Mortuary Affairs Operations, V6.7”, October 2008 (ICDMAO V6.7, 2008). Use of ionizing radiation provides a means of sterilization for which there can be the highest confidence that no viable disease causing organisms survive and minimize risks to mortuary personnel and the environment. Due to the high penetration through biological materials biological agents would be killed even in interior body areas, such as the lungs, abdominal cavity, and gastrointestinal track. No surgical opening or penetration (trochors) would be necessary to introduce materials into these tissue cavities, therefore reducing the possibility of aerosols and minimizing hazards to personnel and the environment. Radiation sterilization is not dependent on an intact vascular system, and therefore the process is applicable to degraded remains as well. Radiation sterilization does not require the use of hazardous chemicals, large quantities of water or specialized temperature, humidity or pressure conditions to be effective. An important recent finding is that military personal protective equipment or standard methods of remains containment do not significantly attenuate the radiation dose (Lowy, Project Experimental Report). Once treated remains would not be subject to further decomposition by bacterial action, as both spore and vegetative forms would be killed. As in mass casualty situations refrigeration is limited and as there are considerable potential health hazards to handling remains this could be an important advantage in using ionizing radiation sterilization (Morgan et al. 2006; ICDMAO V6.7, 2008).

B. Project Goals:

The overall goal of this project is to provide additional information to fill in technical the gaps to increase the TRL rating for the application of this technology to reach TRL6.

The specific goals of this project are:

Execute additional experimental work on radiation inactivation of vaccinia virus, to extend the previous feasibility study demonstrating the ability to inactivate *Bacillus* spores.

Provide on the state of the art report on irradiation technology for inactivation of microbial pathogens as it pertains to development of a transportable system for decontamination of human remains

Provide an assessment of the technical readiness level for a development of a transportable radiation based system for human remains.

Two reports have been provided to addressing the first two goals to the DTRA program office. This document addresses the third goal.

C. Technical Readiness Level Summary

TRL: 5

Biomedical Device TRL: 4

Project TRL: 3

In reviewing the technical level readiness (TRL) literature the Biomedical device standards and the Project Integrations standards (DHS. 2009) were also deemed applicable in addition to the “standard” definitions and rankings for them have been included.

The primary reason that radiation based technology TRL remain below 6 is the specialized characteristics and requirements in applying this widely used technology for use by the Department of Defense Joint Mortuary Affairs community. These characteristics are:

It is necessary to neutralize biological agents of concern include BW/BT threat agents in addition to vegetative bacteria responsible for decay.

Need for a transportable system, capable of being deployed worldwide, and operated in a wide variety of physical environments.

If radiation sterilization alone is considered, including for possible biowarfare/bioterrorism (BW/BT) agents, then it is possible to assign a much higher TRL, possibly TRL 9.

Radiation facilities are currently already in use for this application. Extensive information on the radiation sensitivity of BW/BT agents have been developed (Lowy, Project Irradiation Technology Report, 2009). Dose attenuation caused by the human body is the major factor as personal protective equipment and remains handling containers have been shown to be minimal by experimentation conducted as part of this and previous projects (Experimental Results Report). Radiation sterilization are in extensive in commercial facilities worldwide to sterilize or sanitize a variety of materials. In the context of medical radiation imaging and oncology treatment highly accurate and precise radiation dose exposures to humans are preformed daily.

Therefore it is primarily the issues associated with engineering designs for a transportable system that meets military requirements that need to be addressed to increase the TRL level. Currently the major limitation in progressing to TRL 6 and greater is the development of a full set of technical standards, for construction and acquisition of a full scale prototype for testing in operationally relevant environments.

Importantly, if a fixed facility located, for example in CONUS at Dover, AFB, combined with safe transport from the mortuary affairs collection point (MACP) were an acceptable alternative to the mortuary community to provide a MADCP (mortuary affairs decontamination collection point) then the TRL level for radiation sterilization could also be higher, as this would likely be very similar to commercial facilities used for other purposes (Block, 2000).

The way forward is to form an integrated process team (IPT) of relevant stakeholders, including end users, relevant technical subject matter experts (SMEs), and regulators, to produce a set of standards to guide acquisition of a prototype. These standards would set the design criterion to guide the subsequent acquisition process and deployment of these devices in theatre.

D. TRL Level Evaluation Details

The overall TRL 5 criterion is “Component and/or breadboard validation in relevant environment “. The inactivation of the two classes of biological threat agents has been completed. The tests were conducted with a bacteria spore and virus types which are recognized stimulants for threat agents. Radiation exposure was done in a relevant environment as it was done using a human phantom with radiation attenuation characteristics of human tissue and the phantom was covered with military specification body armor, helmet, body pouch and aluminum transfer case. The radiation characteristics matched those which could be produced by field ready sources (Lowy & Gronemus, Project Experimental Report, 2011).

The Biomedical TRL 4 decision criterion is “Proof-of-concept and safety of candidate devices/systems demonstrated in defined laboratory/animal models.” This is met by the experimentation described in the current project reports and literature cited (see references) and by the extensive commercial use of radiation for sterilization. The Biomedical TRL 5 definition requires testing of in relevant tissue, organ or animal models. This has been completed within the current program. The definition also requires completion of identification of component suppliers and the production and documentation of device component designs and test results. This is beyond the scope of the current project approved goals and funding and has not been completed.

The Project Readiness Level 1 criterion is “Identification of basic scientific concepts and Performers” and this has been completed. The PRL 2 criterion is “Establishment of program with identified customer and technology.” The customer is known, being the DoD mortuary affairs community and radiation sterilization is the technology. The PRL 3 criterion is “Program risk, requirements, and performance characteristics and measures are determined”, which have been described by ICDMAO V6.7, 2008. The PRL 4 criterion is “Integrated Product Teams and working groups for developing and transitioning technology are established”. An IPT has not been established for use of this technology and is identical to the recommended way forward

as described in the initial project proposal and subsequent reports submitted for this project (Lowy, Project Irradiation Technology, 2009).

E. Summary of Radiation State of the Art

The following statements can be made about the state of the art for the use of ionizing radiation for sterilization of human remains:

1. Data on the radiation sensitivity of many microbial pathogens, including possible biological threat agents, are now available.
2. The technological base is well developed for commercial irradiation of a wide variety of materials to effect sterilization.
3. Radiation is currently successfully being used to decontaminate selected portions of the U.S. Mail.
4. The approach has been validated for the inactivation of vaccinia virus and *Bacillus* spores in a human phantom covered in body armor, placed in a body bag inside a simulated Ziegler case.
5. The Armed Forces Epidemiologic Board determined that this approach met the military and Center for Disease Control requirements for return of remains contaminated by *Bacillus anthracis*.
6. Dosimetry measurements demonstrate that personal protective equipment and standard containment used for handling remains have very little effect on the radiation dose delivered to deep tissues. Therefore sterilization can be accomplished without removal of protective equipment or opening body pouches or "Stryker" cases.
7. High energy electron source could fulfill the total radiation dose, dose rate requirements of a capable of biological decontamination of materials including mortuary remains. There are commercially available high energy electron-producing machines that are small and robust enough to be transported. These machines offer the important advantage that when not turned on, no radioactivity is available to pose a hazard or require security for radioactive materials.
8. Equipment could be incorporated within the decontamination line systems as described for Mortuary Affairs Decontamination Collection Point (MADCP) operations.
9. Remains handling methods, such as roller tables, already in use by MADCP could be used for movement, to, through, and from the radiation source.
10. Radiation decontamination could be used on remains regardless of their condition or integrity.
11. Radiation sterilization does not require harmful chemicals or large quantities of water.
12. Remains treated and kept in close containers would not require cooling/ refrigeration as all microbial based decay would be stopped.
13. Radiation sterilization would reduce or eliminate the biological hazards to personnel prior to additional chemical or radiological decontamination. Dose of radiation to inactivate biological agents

are unlikely to affect chemical agents. Therefore no change in the methods for chemical or radiological decontamination would be necessary.

14. Radiation sterilization could reduce the complexity of the required demonstration of decontamination efficacy. In commercial sterilization biological testing is not required as dose measurements showing the dose required was delivered are considered adequate for quality control and assurance. Highly accurate radiation measurements can be done on line and much more easily than microbial viability testing in field environments.
15. Methods and requirements for shielding, monitoring and quality control for ionizing radiation equipment are well established.
16. Safety standards and methods for personnel exposure monitoring using such equipment is well established.

F. Path Forward for Using Ionizing Radiation to Decontaminate Human Remains

Applying the well established technology of ionizing radiation disinfection/ sterilization to the specific application of human remains involves resolving questions in the following areas. It is these questions that an IPT needs to provide specific guidance, which would form the basis of the detailed design criterion for a prototype device.

- First is establishing the specific total ionizing radiation dose necessary.
- Second is defining the operational needs based on the requirements of the mortuary community.
- Third is establishing the type of radiation source and specific engineering design to meet both the treatment efficacy and deployment requirements of a usable real world device.
- Lastly is to identify the regulatory authorities for the development and approval of this application and those responsible for the later oversight and regulation during its actual use.

All of these aspects are those routinely addressed during the application of ionizing radiation sterilization technology in an industrial setting, either when there is a new user of an existing application or there is a new application or configuration for radiation based processing (Block, 2000).

The most recent novel application of radiation based disinfection is the treatment of the U.S. Mail prior to delivery. The methodology was established relative quickly and implemented over the course of several months. The participation agencies in this effort included the U.S. Postal Service, the Armed Forces Radiobiology Research Institute, National Institute of Standards and Technology, and the Food and Drug Administration, all in collaboration with the Office of the Scientific Advisor to the President.

G. Possible Limitations on Current Data

No data was found on the cosmetic effects of ionizing radiation on human remains. Radiation in living individuals can cause burns, but this is due to a complex physiological response which occurs hours, days and weeks after ionizing radiation exposure. These processes presumably requiring live dermal tissues. A small number of reports for sterilization of skin were found, indicating minimal effects (Lowy, Project Irradiation Technology Report, 2009).

The parameter space for radiation inactivation of microbial pathogens is quite large and many of the biological, environmental and radiation quality effects on microbial inactivation are known and now documented within other DoD programs. Especially pertinent is the Empirical Lethality program of the USAF at Kirkland AFB. In principle it is possible the mortuary community would know of specific pathogens or remains conditions that would be need to be evaluated as to the efficacy of radiation sterilization. This possibility was investigated by extensive literature searches and no such information was found (Lowy & Gronemus, 2011, Project Experimental Report).

H. Comment on Equipment Feasibility

In association with a previously request by the mortuary affairs community (Lowy, Program Irradiation Technology Report, 2009) estimates were made of the source characteristics for this application based on the available commercial off the shelf high energy electron sources available at that time. The size and weight of the source and electrical power generator were constrained by transport needs. The weight and size proposed were restricted to be consistent with the source and power supply each being able to be transported by air on standard sized pallets used by the Services and on the ground by a 2.5 ton truck ("deuce and a half") or similar transport vehicle. The tradeoff is higher treatment rates require higher dose rates which require equipment which is comparatively larger. Those which could meet the size and weight requirements for transportability ranged in power levels which were approximately between 3 kW and 1000 kW. Taking into account dose rates it was estimated the number of remains that could be treated per hour ranged from 3 to 20 individual remains from the lowest to highest source power levers. This was based on information about the relationship between power, X-ray conversion, resulting dose rate, and at total required dose of 120 kGy. Current Joint Publication 4-06 Mortuary Affairs in Joint Operations, July 2006 documents indicate that the target rate for MADCPs is decontamination of up to 48 individual remains in 12 hours. Therefore even the smallest units at that time could nearly reach this speed of treatment. The proof of principle studies used total doses which were approximately half of the dose used for these calculations, which would result in estimated treatment rates of 24 to 480 per 12 hours. Since the initial analysis there are likely to have been improvements in equipment weight/ capability and changes in transportation capabilities. An important task for an IPT is to re-evaluate these estimates.

I. Comment on the "Initial Capabilities (ICD) for Mortuary Affairs Operations " Document, October 2008

Within this report an important concern is the further degradation of remains even after treatment at the mortuary MADCP. As discussed above radiation sterilization in principle would eliminate this problem as well as the need for extended refrigeration and the associated equipment.

Table 6-2 Analysis of Material Capabilities for ionizing radiation lists that there would be damage to human remains. This needs to be reconsidered as there seems to be no published information on effects to non-living tissues.

Reference Materials & Literature Cited

Block, S.S., editor. 2000. Disinfection, sterilization, and preservation, 5th ed. Lippencott Williams & Wilkins, Philadelphia.

Department of Homeland Security Technology Readiness Level Calculator (ver.1), Final Report and Users Manual, September 30, 2009.

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Lowy, R.J., Project Irradiation Technology “White Paper” Report, “Current State of Technology Relevant to Development of a Transportable System for Treatment of Contaminated Human Remains”, DTRA Capability Area: Biological Agent Neutralization, Project Number: CA09DEC015, August 2009.

“Mortuary Affairs in Joint Operations”, Joint Publication 4-06, Army Quartermaster, Department of Defense, July 2006.

Morgan OW, Sribanditmongkol P, Perera C, Sulasmi Y, Van Alphen D, et al. (2006) Mass fatality management following the South Asian tsunami disaster: Case studies in Thailand, Indonesia, and Sri Lanka. PloS Med 3(6): e195. DOI: 10.1371/journal.pmed.0030195

Technical Readiness Assessment Deskbook, Department of Defense, September 2003.

Technical Readiness Assessment (TRA) Deskbook, Department of Defense, July 2009.

APPENDIX

Technical Readiness Definitions Used

Levels judged completed are shown in bold.

TABLE 1
Technical Readiness Assessment (TRA) Deskbook, Department of Defense, July 2009

TecTable C-1. Hardware TRL Definitions

	Definition
1	Basic principles observed and reported
2	Technology concept and/or application formulated
3	Analytical and experimental critical function and/or characteristic proof-of-concept
4	Component and/or breadboard validation in laboratory environment
5	Component and/or breadboard validation in relevant environment
6	System/subsystem model or prototype demonstration in a relevant environment (ground or space)
7	System prototype demonstration in a space environment
8	Actual system completed and —flight qualifiedII through test and demonstration (ground or space)
9	Actual system —flight provenII through successful mission operations

TABLE 2
Table E-1. Proposed TRLs for Medical Research, Development, Test, and Evaluation (RDT&E)

	Definition	
1	Basic principles observed and reported	Lowest level of technology readiness. Maintenance of scientific awareness and generation of scientific and bioengineering knowledge base. Scientific findings are reviewed and assessed as a foundation for characterizing new technologies.
2	Technology concept and/or application formulated	Intense intellectual focus on the problem, with generation of scientific “paper studies” that review and generate research ideas, hypotheses, and experimental designs for addressing the related scientific issues.
3	Analytical and experimental critical function and/or characteristic proof-of-concept	Basic research, data collection, and analysis begin in order to test hypothesis, explore alternative concepts, and identify and evaluate component technologies. Initial tests of design concept and evaluation of candidate(s). Study endpoints defined. Animal models (if any) are proposed. Design verification, critical component specifications, and tests (if a system component or necessary for device test and evaluation (T&E)).
4	Component and/or breadboard validation in laboratory environment	Non-GLP laboratory research to refine hypothesis and identify relevant parametric data required for technological assessment in a rigorous (worst case) experimental design. Exploratory study of candidate device(s)/systems (e.g., initial specification of device, system, and subsystems). Candidate devices/systems are evaluated in laboratory and/or animal models to identify and assess potential safety problems, adverse events, and side effects. Procedures and methods to be used during non-clinical and clinical studies in evaluating candidate devices/systems are identified. The design history file, design review, and, when required, a Device Master Record (DMR), are initiated to support either a 510(k)4 or Premarket Approval (PMA).
5	Component and/or breadboard validation in relevant environment	Further development of selected candidate(s). Devices compared to existing modalities and indications for use and equivalency demonstrated in model systems. Examples include devices tested through simulation, in tissue or organ models, or animal models if required. All component suppliers/vendors are identified and qualified; vendors for critical components are audited for cGMP/Quality System Regulation (QSR) compliance. Component tests, component drawings, design history file, design review, and any DME are verified. Product Development Plan is drafted. Pre-Investigational Device Exemption (IDE) meeting is held with Center for Devices and Radiological Health (CDRH) for proposed Class III devices, and the IDE is prepared and submitted to CDRH.
6	System/subsystem model or prototype demonstration in a relevant environment	Clinical trials are conducted to demonstrate safety of candidate Class III medical device in a small number of humans under carefully controlled and intensely monitored clinical conditions. Component tests, component drawings, design history file, design review, and any DMR are updated and verified. Production technology demonstrated through production-scale cGMP plant qualification.
7	System prototype demonstration in an operational environment.	Details not relevant for this Report

8	Actual system completed and —flight qualifiedII through test and demonstration	Details not relevant for this Report
9	Actual system —flight provenII through successful mission operations	Details not relevant for this Report

TABLE 3

Department of Home Land Security Project Readiness Level Definitions

Table 4: DHS S&T RL Calculator (Ver1.1) high-level PRL definitions PRL	PRL Definition
1	Identification of basic scientific concepts and Performers.
2	Establishment of program with identified customer and technology.
3	Program risk, requirements, and performance characteristics and measures are determined.
4	Integrated Product Teams and working groups for developing and transitioning technology are established.
5	Systems engineering methodology, system architecture and end user involvement are established.
6	Formal requirement documents, final Test and Evaluation Master Plan, and Systems Engineering Plan are complete.
7	Finalized Verification, Validation and Accreditation of system.
8	Training and Test and Evaluation Documentation are complete.
9	Safety and Training is complete.

Contaminated Human Remains: Transportable Decontamination Vaccinia Virus Ionizing Radiation Inactivation in a Human Phantom

Project Experimental Report

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Summary

This report summarizes the experimentation done with vaccinia virus and a human phantom to provide proof of principle data that ionizing radiation can inactivate an important threat agent within human mortuary remains. Vaccinia virus (VV) Lister strain was used as a stimulant for *Varola major* the causative agent of smallpox. The experimental method was similar to the one used previously to verify ionizing radiation could inactivate *Bacillus anthracis*, in human remains. Those experiments were reviewed by the CDC mortuary board and it was concluded that human remains contaminated with *Bacillus anthracis* could be returned to CONUS safely after radiation sterilization.

The approach was to use a human phantom which has sample positions throughout its volume. The human phantom is a plastic manikin with varying density to mimic the heterogeneities with the body which can alter the radiation dose actually absorbed at particular locations. Vials were placed into the sample positions containing a biological target or alanine pellets to measure the dose of ionizing radiation.

The phantom was exposed to radiation using several configurations to account for the presence of materials which would shield the microbiological targets from ionizing radiation (See Appendix 2). The first of these configurations was with the phantom uncovered. The second the phantom was wearing the currently available torso vest body armor and placed in standard plastic body pouch used for human remains. The third configuration was the same as the second with the addition of an outer aluminum case. The case was constructed of the same alloy and thickness as human remains transfer cases (Stryker case). These three configurations were used to construct a map of the radiation dose rates at 12 different points throughout the volume of the phantom. Based on these measurements the external dose sufficient dose to kill vaccinia virus at all locations with the body phantom was calculated. A fourth configuration with the phantom wearing the body armor and a helmet was used to determine the dose rate to the head.

The human phantom with samples of vaccinia virus was exposed using two configurations. The first was the phantom in the body pouch. The second the phantom was wearing the body armor, in the pouch all enclosed within the external aluminum case. The target total minimum radiation dose was 30 kGy, which other studies had shown should full inactivate the virus. The external dose was 42 and 52 kGy respectively for these two configurations. Analysis of the biological samples showed that the virus was killed at all locations throughout the phantom.

Therefore this proof of principle study combined with previous experiments shows that ionizing radiation can effectively decontaminate human remains. It is capable of doing so even when those remains are contained within the standard types of containment used for handling and standard military protective equipment is in place.

This study provides additional information. The dosimeter maps demonstrated that none of the external materials had large shielding effects, e.g. the helmet, body armor and case caused little attenuation of radiation dose. The major dose reduction factor was the human phantom itself. Two additional small studies were completed. Post death internal body fluid composition is altered to high potassium. Radiation inactivation experiments showed this change in ionic composition did not alter virus radiation sensitivity. Forensic analysis is important; both to determine the type of infectious agent causing death and remains

identification. Many analytical techniques are based on DNA technologies and it would be advantageous if these could be applied after infectious disease hazards were eliminated. Shown here is the high radiation doses do affect the ability to polymerase chain reaction methods. It appears, depending on the dose and target, these identification methods may or may not be impaired, and further investigation is warranted.

Background Information

The objective of this project is to support the developmental process of a deployable field ready system to decontaminate human remains using ionizing radiation.

The inability to safely return biologically contaminated remains to the families of service members who died in defense of their country has been a persistent problem. A number of approaches have been considered, but all have problems and short falls. These concerns have been reviewed and documented in “Initial Capabilities Document for Mortuary Affairs Operations, V6.7”, October 2008.

Use of ionizing radiation provides a means of sterilization for which there can be the highest confidence that no viable disease causing organisms survive and minimize risks to mortuary personnel and the environment. Due to the high penetration through biological materials biological agents would be killed even in interior body areas, such as the lungs, abdominal cavity, and gastrointestinal track. No surgical opening or penetration (trochors) would be necessary to introduce materials into these tissue cavities, therefore reducing the possibility of aerosols and minimizing hazards to personnel and the environment. Also sterilization is not dependent on an intact vascular system, and therefore the process is applicable to degraded remains as well. Once treated remains would not be subject to further decomposition by bacterial action, as both spore and vegetative forms would be killed. Likely most or all fungus growth would also be eliminated as their radiation sensitivity. The majority of vegetative and fungus radiation sensitivities are greater than bacterial spores and viruses, with a few types having nearly identical sensitivity. As in mass casualty situations refrigeration is limited and there are considerable potential health hazards to handling remains (Morgan et al. 2006) this could be an important advantage in using ionizing radiation sterilization.

Application of Ionizing Radiation to Human Remains

Applying the well established technology of ionizing radiation disinfection to the specific application of sterilization of human remains involves revolving questions in the following areas.

- First is establishing the specific total ionizing radiation dose necessary.
- Second is defining the operational needs based on the requirements of the mortuary community.
- Third is establishing the type of radiation source and specific engineering design to meet both the treatment efficacy and deployment requirements of a usable real world device.
- Lastly is to identify the regulatory authorities for the development and approval of this application and those responsible for the later oversight and regulation during its actual use.

All of these aspects are those routinely addressed during the application of ionizing radiation sterilization technology in an industrial setting, either when there is a new user of existing applications or there is a new application for radiation based processing.

The goal of this study was to further establish the total ionizing radiation dose necessary.

This study demonstrates that the previous information on vaccinia virus radiation sensitivity could be applied to material contained with a human body resulting in complete inactivation.

Radiation sensitivity values for a broad variety of bacteria, bacterial spores and viruses is available from the open literature studies. However the conditions of exposure and the specific agents used are primarily those of concern for food and medical devices. Much of the available data does not address the radiation sensitivity of possible threat agents or a broader set of radiation exposure conditions or physical and chemical conditions, temperature, and wetness or dryness, that might be encountered the field environments where inactivation of microbial agents might need to be done for decontamination of remains. This laboratory has been actively engaged in filling these information gaps through other research projects. The information from these studies was used as a basis to determine the radiation dose necessary to inactivate the virus. The major difference in this study was the target organism was in a simulated human body.

Methods In Brief

Two strains of vaccinia virus have been used in this, and extensively in other studies of radiation sensitivity by this laboratory, Western Reserve (WR) or Lister. Lister has been used in humans, as a vaccine, despite having limited pathophysiology in some individuals receiving the vaccine. WR is a more attenuated laboratory strain, used for research applications, but is not used as a vaccine due to low human immune response. The experimental goal was to apply previous known pathogen sensitivity values to the measured phantom dose rates to determine total dose and demonstrate complete biological inactivation when samples were exposed within the phantom. Therefore the experimental questions could be answered using either strain. The small scale experiments were conducted with the WR strain of vaccinia virus as from other studies there is more information on how physical-chemical conditions alter this strain's radiation sensitivity. The decision to use the Lister strain for the phantom exposures was primarily a practical one. During the course of these studies there was a 60% increase in source time costs. The previously determined dose for full inactivation of the Lister strain was known to be lower than for WR. Therefore for the phantom experiments this strain was chosen as a means of keeping these experiments within budget. The use of the more in vivo virulent strain was an additional benefit.

Detailed methods for virus preparation, the human phantom dimensions, exposure configurations and photographs showing the phantom experimental configuration are in Appendices 1 to 3.

In brief vaccinia viruses were produced by standard methods using cultured cell lines. Cell lysates without further concentration or purification were used. The presence of cell debris and culture media residues is more consistent with the conditions surrounding the virus if in actual body tissues. Biological components can act to both stabilize the virus and to protect against radiation damage. Virus preparations were used both in the fully hydrated state and as dried material. Dry material was used for the human phantom exposures as previous studies suggest that for ambient temperature this form has the greatest radiation tolerance. Plaque

assays were performed indicating that sample preparative processing, including thawing, heat sealing, and refreezing had no effect on viral titer (Appendix 6).

Vials containing either dosimeters or virus samples were placed in known locations throughout the phantom as shown schematically in Figure 1 and Appendix 2. Radiation exposures were conducted in the AFRRRI cobalt-60 facility using the maximum dose rate available which is approximately 6.5 kGy. It is expected that any device developed would use as high dose rates as possible to maximize the speed of treatment.

Preliminary exposures were done with the human phantom only containing alanine dosimeters to determine dose rate: 1) uncovered bilateral exposure, 2) uncovered unilateral exposure, 3) covered with the Improved Outer Tactical Vest (IOTV) that contained forward and rear Enhanced Small Arms Protective Inserts inside the human remains pouch, 4) with all covering materials described in number 3 all enclosed within the aluminum case and 6) covered with the vest, plates, and the head with the Advanced Combat Helmet. These experiments provided information on the radiation attenuation characteristics.

Experiments with virus samples were done for two of the above configurations, using a total internal target dose of 30 kGy. The external dose used was based on the lowest dose rate value obtained within the phantom. The first irradiation scenario was gamma exposure for the human phantom covered in a government spec human remains pouch (Figure 2-3). The second scenario was virus was irradiated in the human phantom with the protective vest and inserts covered in the body pouch enclosed in the aluminum case (Figure 2-4C). Following exposures the virus samples were stored at -80°C until the titer was determined by plaque assay.

Results and Discussion

Phantom Exposure Dosimetry Results

Table 1 and Figure 1 summarize the dosimetry rate measurements. The measurements were used for two purposes. First it provided information on how much the human body, personnel protective equipment, and containers for remains alter the radiation dose received at various positions within the human torso. The second use of the data was to determine the total exposure time when exposing the phantom when the VV biological samples were in place.

Table 1

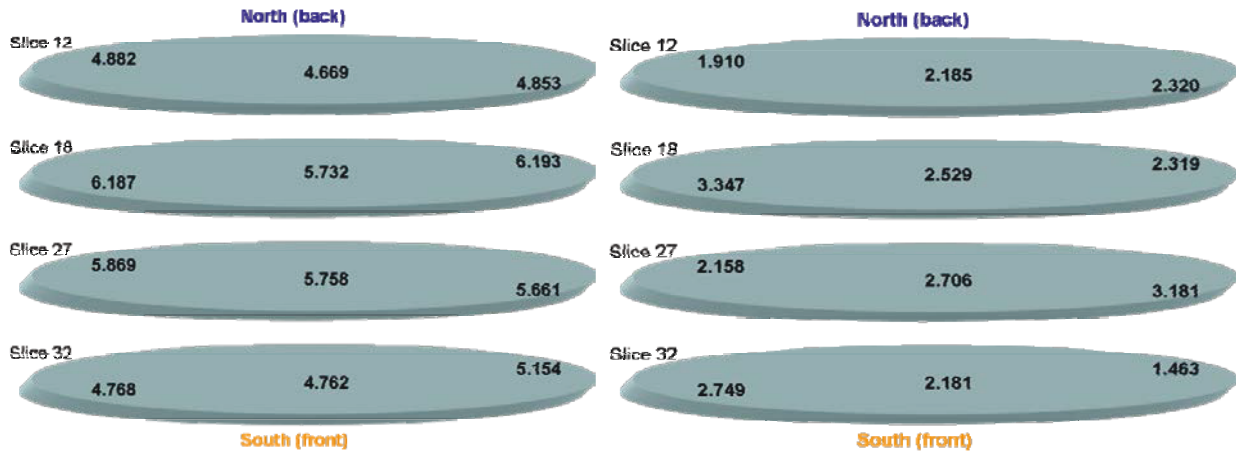
Phantom Dose Rate Summary

Averaged Dose Rates for All Positions – kGy / h				
Exposure	Bilateral			Unilateral
Phantom Configuration	Uncovered	Vest and Pouch	Vest Pouch Case	Uncovered
Average:	5.37	4.81	4.71	2.42
Min:	4.67	3.87	3.77	1.46
Max:	6.19	5.43	5.33	3.35
Range: ±	14.19%	16.22%	16.58%	38.90%
RSD:	10.84%	10.96%	11.12%	21.63%

Table values are the average for all the measurements for all positions in all slices. Shown is the average, the maximum value and minimum value, the per cent variation and per cent root mean square. Note that three phantom configurations were done with bilateral source configuration. The uncovered configuration was also performed with a unilateral exposure. The free in air dosimetry rate for bilateral and unilateral exposure were approximately 200 Gy at a dose rate of approximately 107 Gy/min

Importantly, these results suggest that protective equipment and mortuary containers have only small dose attenuation effects. Therefore the major reduction in dose is due to the phantom (human body) density itself. For the uncovered phantom the lowest dose rate observed was 4.67 kGy/h, which is 72% of the free in air dose of 6.5 kGy/h. The exposure condition having the greatest attenuation was when the phantom was covered with the vest, pouch and case. The lowest dose rate observed in this configuration was 3.771 kGy/h is 81% of the dose rate without protective equipment or 58% of dose rate free in air. The dosimetry for the head shows no attenuation as the rate was 3.16 kGy/h without the helmet, and 3.20 kGy/h with it in place, a difference within analytical measurement variability. Therefore, addition of equipment caused, on average, only an additional 20% reduction in dose rate. This suggests that a successful decontamination process could be developed without the necessity for removing clothing and most equipment from the remains. This could reduce the time for handling individual remains, allowing the overall process to be more rapid, and reduce hazards to mortuary personnel.

Figure 1 - Summary of Phantom Dose Rate Measurements

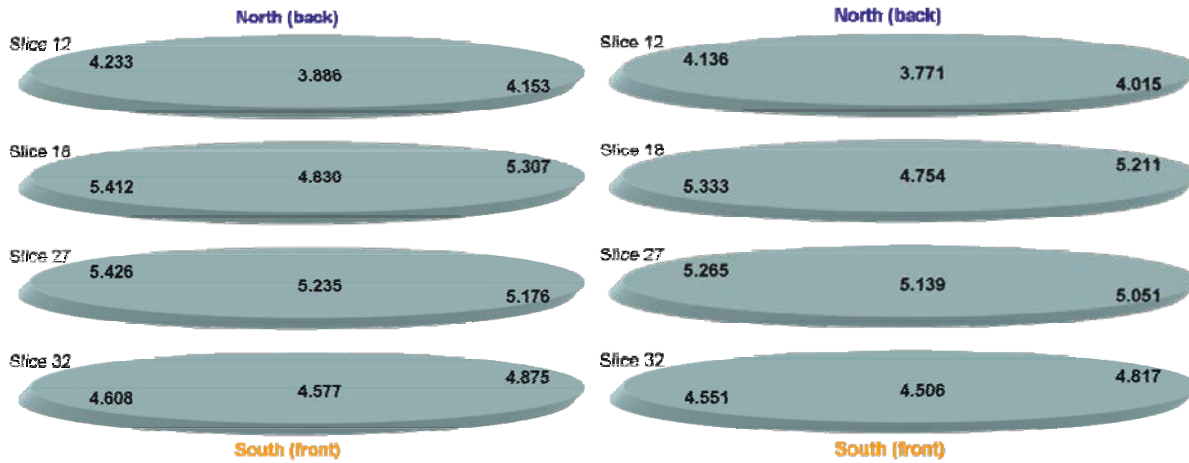


Uncovered – Bilateral Exposure

Uncovered - Unilateral Exposure

Vest and Pouch – Bilateral Exposure

Vest, Pouch, and Case – Bilateral Exposure



Schematic diagram the radiation dose rate in kGy/h) measured at each of position in the phantom under the different exposure and shielding conditions as indicated by the captions. Note that the left and right most positions within each slice are not along the left to right (caudal) center line but are at different depths relative to the front (anterior) surface. For example the in Slice 12 of the uncovered phantom the right most dose rate (4.853) was measured at a position closer to the anterior surface than the left most data value (4.882), which is closer to the posterior surface. These depths alternate between the slices. The central positions the approximate geometric center of the slice, e.g. at the greatest distance from the surface in all directions. The exposure facility sources are indexed according to the cardinal compass points. The front of the phantom was facing the south cobalt array and the back was towards the north cobalt array.

The data also show that the dose rate heterogeneity throughout the body is relatively small, being about 16% or 1.56 kGy/h difference from the lowest to highest rate. However this value is somewhat misleadingly overly large as the lowest dose rates values are from the middle sample of slice 12 and the head, approximately 3.8 kGy/h. Both of these positions due to the geometry of the phantom and radiation field are well out of the direct line of site of the source (Appendix 3 Figure 1 and 2). A better comparison to evaluate the heterogeneity caused by the phantom and equipment is to use only the dose rate values from slices 18 to 32

as these are directly opposite and completely within the radiation field. The range for the vest, pouch, case setup is 5.33 to 4.51 kGy/h a difference of only 0.82 kGy/h.

Bilateral exposures were primarily used in this study as this provides the highest dose rate, the most uniform field and most efficient use of exposure time. Despite using a very even radiation field the total doses values differ by about 10 kGy. This is because even small dose rate changes are accentuated by the high to total doses needed. For the phantom with all the covering materials the range is 30.5 to 42.5 with a 11 % coefficient of variation. Another factor relative to total dose variation is the difference between bilateral and unilateral sources. Measurements for unilateral exposure were performed, as it is possible that the final configuration of radiation source equipment would use a unilateral source. The unilateral exposure results illustrate that the effect is not simple, as the actual dose rates are not simply 50% of the dose rates for bilateral exposure at each of the corresponding positions. Also the magnitude of per cent change does not correlate with the magnitude of the rate or position. The unilateral dose rate values average 44.6% of the bilateral ones, ranging from 28% to 53%. The use of a unilateral source also increases the variability of the dose rates as shown by the approximately doubling of the RSD and Range value.

This greater heterogeneity of dose when using a unilateral source, especially when exposing an object like the phantom with variations in density, is a well known phenomenon. Simplistically, fewer source elements and gamma photons coming from only one direction make for a less uniform field and the reflections and refraction of photons caused by the target result in further changes in the dose map, resulting in high and low dose regions. Use of a bilateral field, with photons entering the target from additional directions tends to even out the regions of dose. An intuitive illustration is what happens when illuminating an object with areas having differences in transparency with one or two light sources, and the resulting change in shadowed areas and their darkness. Importantly these effects of dose rate variation can be effectively addressed as illustrated by the biological inactivation results.

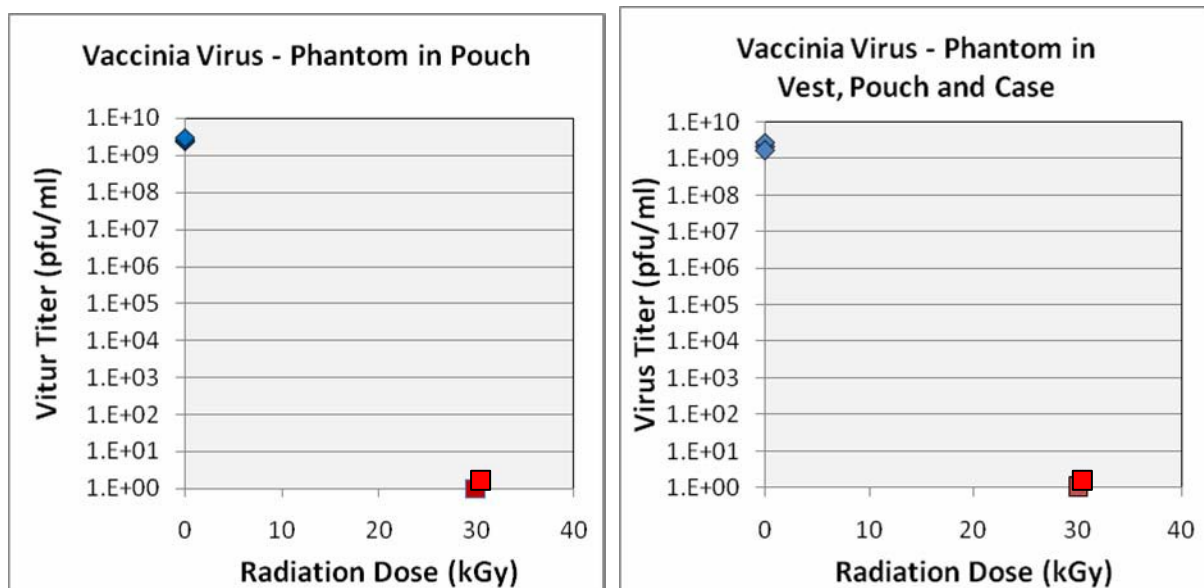
The second use of these dose rates was to plan the total dose necessary for inactivation of the VV biological indicator. Based on previous radiation sensitivity measurements for dry, crude preparations of the Lister strain a target of 30 kGy was chosen. For each the two configurations used to expose VV samples the total time was set by the lowest dose rate. As the pouch is composed of light plastic materials, which have low radiation attenuation properties, the dosimetry measurements for the uncovered phantom were used. The lowest minimum dose rate value was 4.6, rounding down to be conservative sets the exposure time of 6.5 hours. For the configuration with the vest, pouch, and case the minimum dose rate was 3.7 kGy for a total exposure time of 8 hours. The source was not available for a continuous exposure, so the exposure was fractionated into two 4 hour time periods. Data from other studies suggested that fractionation would not alter the radiation sensitivity of the virus. Applying the total time based on the lowest dose rate to the dose rate values for each position results in the range of approximately 30 to 42 kGy. Therefore the prediction was that was no sample position that would not receive a dose sufficient to fully inactivate the virus.

Phantom Exposure Biological Results

Figures 1 and 2 show the results for the virus exposed inside the phantom without any external equipment and with the vest, pouch and aluminum case. For both exposure conditions no viable virus was recovered at any position (Appendix 4). This confirms that the dose calculation predictions were correct. The plots all of the values for both the unirradiated controls (n=3) and the exposed (n=12) showing how closely individual values overlay one another. In this particular set of experiments the total reduction in infectious virus levels is 9.3 $\log_{5_{10}}$.

Figure 2

Summary of Virus Titers - Samples Exposed to Radiation within the Human Phantom



These results show that for this application microbial radiation sensitivity values determined previously combined with a dose rate radiation map determined by physical measurements together can be successfully predict the total dose necessary for this application to treatment of human remains. The results further show that ionizing radiation can fully inactivate virus within a human body analog. Furthermore, typical military personnel protective equipment does not need to be removed prior to radiation sterilization, when the appropriate adjustment for dose rate attenuation is considered. Likewise human remains, with protective equipment, can be treated without removal from either body pouches or the heavier aluminum cases used to contain and handle human remains.

A significant, rise in the core temperature to 29°C or 33 °C for the two phantom experiments with virus samples occurred. Temperature rise occurs due to the absorption of radiation energy. The rise here of up to about 3.7°C per hour for these high doses and dose rates is consistent with observations from other

experiments measuring the temperature rise in water. The extent of rise of course is dependent on the external temperature, which in all studies is approximately 22 °C. Therefore it is reasonable to expect that amount of heating would occur for human remains, especially if desiccation has not occurred. However, the temperature rise is low enough that it is unlikely that it contributes significantly to inactivation of the virus. However, this change in temperature could have impact on the condition of the remains or handling procedures which should be considered.

Additional Biological Experimentation Results

Two additional experiments were performed using vaccinia virus. The first was changing the ionic environment of the virus and the second was to determine the utility of using PCR identification based methods post high dose radiation exposure.

High Potassium Experiments

The type of radiation, microbe characteristics and the physical chemical environment potentially can change the radiation sensitivity of microbial pathogens. The effect of these parameters has been addressed and reviewed extensively and most recently in the context for DoD interests. In reviewing what was known about human remains (Dent et al. 2004; Madea, 2005) a striking difference was immediately post death is a change in the ionic composition of body fluids from low potassium to a high potassium environment (Madea, 2005; Querido, 1991). This is likely due to release of potassium from cells to body compartments; the normal physiological state is high sodium low potassium in blood and body fluids, but the reverse within cells.

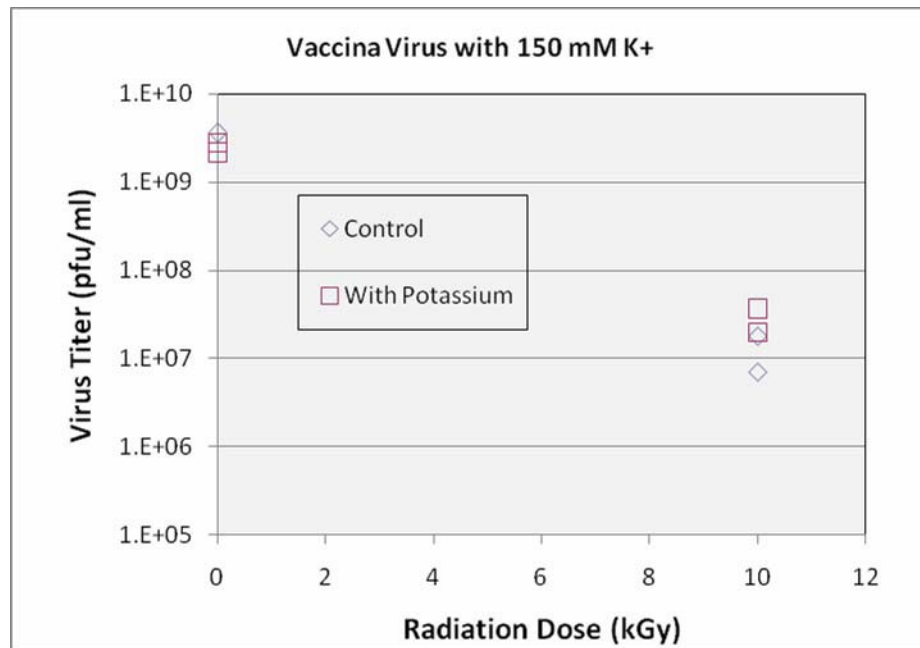
The potassium concentration in stock hydrated vaccinia virus preparations was increased to 120 mM. The virus was exposed in the fully hydrated state. The virus preparation was unpurified, with the virus particles in a crude lysate of the cells used to grow the virus. Therefore in addition to viral particles this suspension contains high levels of cell debris, soluble proteins, small biological molecules, and buffered physiological saline. In effect this experiment mimics the expected conditions for remains post-death prior to any desiccation. The radiation dose was chosen purposefully as one which would only partly reduce virus titer, so that the treatment effects on radiation sensitivity could be compared.

As shown in Figure 4 the high K did not significantly alter the radiation response as the irradiated sample titers are overlapping and the small median difference, while possible a real effect, is within variation for the titer assay alone.

No specific information was found in published literature on other changes in the physical chemical environment immediate post-death or prior to internment, such as pH change, release of specific chemicals or enzymes, which might have effects on microorganisms within remains. There are limited reports on changes in nitrogen compounds (Zhu et al. 2007) and small acidification changes in animal tissue (Henckel et al. 2000). If the pathology or mortuary community provides additional information, effects of those changes on radiation sensitivity may need to be addressed to optimize sterilization processes. The postmortem literature does comment that under some internment conditions a large amount of saponification occurs to soft tissues (Dent et al. 2004; Forbes et al. 2005). This process converts tissue to a waxy soap like material. As the conditions that cause this change are high in water content they are expected to be detrimental to most

viruses' survival and be detrimental or have no effect on spores, but no published studies have been found on how this process alters infectious agent presence or viability. For radiation sterilization it will change the body density; if as expected a density less than intact tissues, the internal radiation dose would be expected to be higher for the same external radiation dose.

Figure 4
Virus Radiation Sensitivity Response for Altered Ionic Composition



Radiation Exposure Effects on PCR Analysis

Polymerase chain reaction (PCR) based methods are widely used for biological identification including assays for the specific type of microbial threat agent present and for identification of humans. There is published data suggesting that bacterial spore identification by antibody and PCR tests can be compromised by various methods of inactivation including ionizing radiation (Dang et al. 2001). PCR and other DNA based techniques dependent on PCR are now extensively used for identification of individuals. However, there appear to be no published studies on the effects of ionizing radiation on the effects of human identification post exposure or for other pathogens. Therefore a small pilot study was conducted.

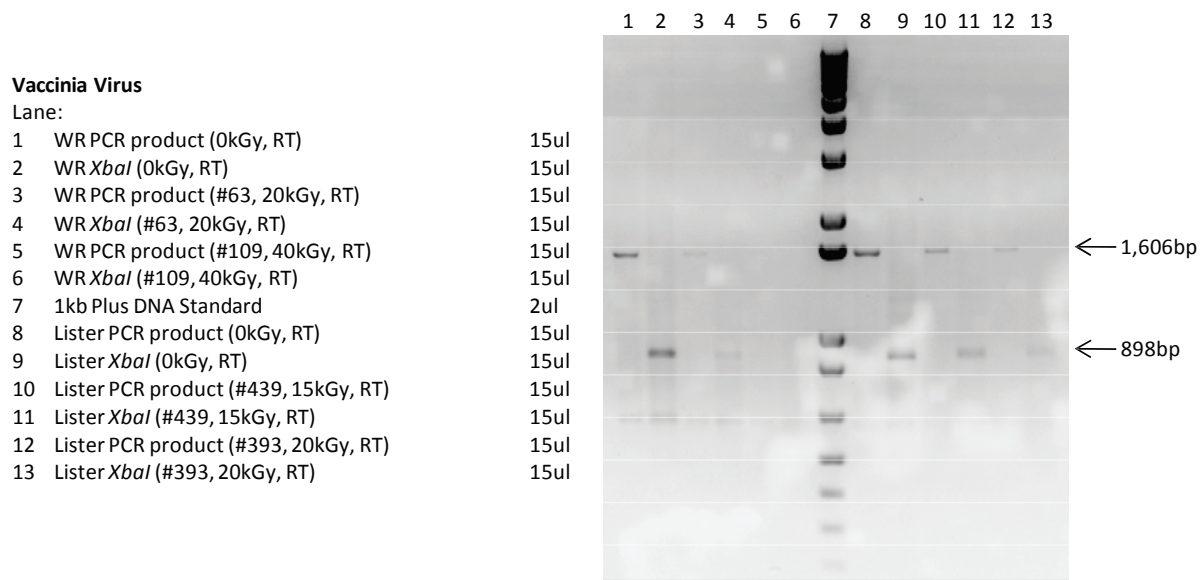
In the context of this project vaccinia virus was readily available to be used for this type of study. It of course is the appropriate stimulant for *Variola major* the causative agent of smallpox. As an agent of concern for bio-warfare and bioterrorism it is one of the microbial pathogens that would be screened for post a mass casualty event. The genome is one of the largest and complex viral genomes, potentially being more difficult

for analysis once damaged. As this was a small pilot study the administrative time and effort to obtain human tissue was not considered appropriate.

Gene targets for vaccine virus identification are well established from the scientific literature. Specifically the N-terminus region of the gene encoding for A-type inclusion protein (ATIP) is highly conserved across many vaccinia strains (DeCarlos and Paez, 1991), therefore primers against this region is widely used for detection. Two strains of vaccinia virus, WR and Lister strains were used. As described, these two strains have different radiation sensitivities. Both strains were exposure to intermediate doses of radiation and high doses of radiation. The intermediate doses correspond to those which result in approximate equivalent levels of survival level for the two strains. The high doses correspond to doses that viable virus cannot be detected by plaque assays. The intermediate doses were 20 kGy and 15 kGy and the high doses were 40 kGy and 20 kGy for WR and Lister respectively.

The data show that a PCR product (Lanes 1 and 8) and endonuclease product (Lane 2 and 9) can be generated for this gene from unirradiated controls of both vaccinia virus strains (WR, Lanes 1 and 2; Lister Lanes 8 and 9). Post exposure to the intermediate radiation dose PCR products and post endonuclease treatment products could also be obtained for both strains (WR, Lanes 3 and 4; Lister, Lanes 10 and 11). However, the bands were less dense, indicating less product was obtained for both strains, with WR being less than Lister. After the high dose exposures these exposures bands can be obtained for Lister but not WR (WR, Lanes, 5 and 6; Lister Lanes, 12 and 13).

Figure 5 – PCR Detection of the ATI Vaccinia Virus Gene Post Radiation Exposure



*Xba*I digestion was used as it was expected to differentiate between Vaccinia strains. According to sequences on PubMed, restriction enzyme digestion of the WR PCR product was expected to produce fragments in sizes 169bp, 216bp, 323bp, and 898bp, while the Lister amplicon should have produced fragments in sizes 69bp, 100bp, 216bp, and 1,221bp. However, *Xba*I digestion performed here produced the same fragments with both WR and Lister. Because the fragment sizes did not differ the effects of radiation exposure on distinguishing between strains could not be assessed. It is possible that this laboratory's WR and Lister strains, derived from ATCC standard stocks sequences do not match those published. Therefore further investigation needs to be performed, such as sequence analysis of the virus stocks used in this study, and use of other restriction enzymes instead of *Xba*I.

The results show that despite exposure to very high doses of ionizing radiation, including those which can fully inactivate many microbial pathogens, that PCR detection assays can be used. However, the further implication is that this is not a certainty, and use of PCR methods for identification of either threat agents or human remains could fail if a sufficiently radiation resistant gene target is not used. Taken together the results for the two strains implies the loss of sufficient material for detection correlated with increasing dose, at 15 kGy there was preservation of the target, at 20 kGy the target was reduced to just detectable levels and at 40 kGy was not detectable. There was not a correlation between complete loss of viability and detection, Lister is completely inactivated at 20 kGy, but ATI could still be assayed. This suggests that alternate gene targets could exist for WR that would allow its detection at higher doses. The reasoning is as it is more radiation resistant, and loss of titer is primarily based on genetic damaged, and therefore there are other genes which would be less damaged at 40 kGy or greater doses, and therefore detectable by PCR. Additional experimentation is warranted to determine whether there are gene targets, especially among those already used for detection, that are sufficiently undamaged by high radiation doses that PCR methods can be applied. Similar studies would need to be conducted for human genes, e.g. genetic "finger printing" targets, to determine which ones can be successful used, and their dose limitations.

Recommendations

It is recommended that this report be provided to relevant Department of Defense mortuary community members and “stakeholders” for their review and consideration.

These results confirm provide proof of principle data that vaccinia virus can be inactivated within human remains. They show that by combining knowledge about the amount of virus, the radiation sensitivity and the minimum dose rate that the total dose necessary for complete inactivation of the target material can be obtained.

Experiments done in a very similar fashion, with a stimulant for *Bacillus anthracis*, when provided mortuary affairs board, resulted in their certifying that radiation sterilization was an effective means for decontaminating human remains from that threat agent. Therefore it seems likely a similar conclusion would be forthcoming for orthopox viruses, e.g. *Varola major*, based on these experiments.

Radiation sterilization is a well established industrial process (Lowy; White Paper 2010). Taken together these studies provide a reasonable basis to conclude that the principles of radiation sterilization are sufficiently well understood to determine the dose or doses of radiation necessary for decontamination of human remains. Therefore it there is a solid proof of principle basis to acquire the additional information necessary to evaluate whether radiation sterilization is a viable real world solution to the decontamination of human remains for military operations.

The information now needed includes, but is not limited to, the requirements of the Department of Defense, the mortuary community, the location sites needed and/or transportability of such equipment, and the specific types of radiation sources devices, and their capabilities, which exist or could be constructed. Details of the type of information and recommendations as to what will be required to increase the technical readiness level of this approach can be found in the previous White Paper and in the final integrated report requested by the program office.

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Appendix 1

Detailed Methods and Materials

Vaccinia Virus Propagation and Preparation

HeLa S3 cells (ATCC #CCL-2.2, American Type Culture Collection, Manassas, VA) were seeded onto five 175cm² tissue culture flasks 18-24 hours prior to infection at a density of 5x10⁷ cells per flask in growth medium (Minimal Essential Medium with Earle's Balanced Salt Solution, 10% non-heat inactivated FBS, 2mM L-glutamine, 50µg/mL gentamicin). All incubations for this cell type and procedure were at 37°C, 5% CO₂. A 1:1 mixture consisting of a 1mL vaccinia virus Lister (ATCC #VR-1549,) aliquot, with a titer of approximately 1x10⁹ pfu/ml, and 0.25mg/mL trypsin from porcine pancreas (Sigma, Inc., Saint Louis, MO) was made. This virus/trypsin mixture was incubated at 37°C for 30 minutes, alternating between vortexing for 10 seconds and sonicating for 30 seconds every 5 minutes. The virus/trypsin was then mixed with 8mL MEM-2.5 (Minimal Essential Medium with Earle's Balanced Salt Solution, 2.5% non-heat inactivated FBS, 2mM L-glutamine, 50µg/mL gentamicin). After washing the HeLa S3 cells twice with PBS, they are infected with 2mL of the virus/trypsin/media mixture for 2 hours, rocking every 30 minutes. Following infection, 25mL of MEM-2.5 was added to each flask for 72 hour incubation. To prepare the viral aliquots for use, the detached infected cells were centrifuged for 5 minutes at 1800xg at 10°C; the supernatant was carefully aspirated off. The pellets were resuspended in 10mL MEM-2.5 and subjected to 3 rounds of snap-freezing and thawing. Lastly, the sample was sonicated for 45 seconds prior to aliquoting 200µl per O-ring sealed tube, snap-freezing, and storing at -80°C.

Vaccinia Virus Drying and Sample Preparation

Crude hydrated virus in 200µl aliquots contained in O-ring sealed tapered 1.5 ml cryovials were thawed on ice for 30 minutes then dried using a SPD121P-115 Speedvac (Thermo Electron, Corp.) until vacuum pressure fell below 30 microns. This took approximately 3.5 hours with temperature remaining between ambient and 35°C. Samples were stored at -80°C until use. Prior to use in experimentation the cryotubes were removed from storage, placed on ice for 30 minutes, then heat-sealed using Cryoflex sheathing (Nunc, Thermo Fisher, Inc.) (Figure 2-1) and returned to storage at -80°C. This provided the equivalent of a primary and secondary containment system that fit within the sample holes within the phantom.

Gamma Irradiation for Dosimetry

Exposures for dosimetry measurements were conducted first with several configurations to determine the dose rate at each sample position. The following equipment was used as their presence results in partial or complete shielding of the phantom and reduction of the absorbed radiation dose: government spec human remains pouch with an envelope zipper (Extra Packaging, Corp., Rochester, NY) (Figure 2-3). Improved Outer Tactical Vest (IOTV; Point Blank Body Armor, Inc. Pompano Beach, FL) containing forward and rear Enhanced Small Arms Protective Inserts (ESPI; Ceradyne, Inc., Costa Mesa, CA), (Figures 2-4a, 2-4b, and 2-4c), an aluminum box constructed of the same alloy and thickness as the remains transfer cases (Stryker case) consisting of 3003-H-14, 0.09 inch thick aluminum (Figures 2-5a and 2-5b), and an Advanced Combat Helmet (ACH, Gentex, Manchester, NH).

The configurations used with were: 1) phantom uncovered bilateral exposure 2) phantom uncovered unilateral exposure 3) phantom with IOTV (vest) and pouch and 4) phantom with IOTV, pouch and aluminum case. Dosimetry measurements from these configurations were used to plan the experiments using virus. A final dosimetry exposure was done with the vest and ACH for head dosimetry only. For each of these

exposures the phantom contained vials with alanine dosimeters and was exposed to a total external dose of 200 Gy at a dose rate of approximately 107 Gy/min (range 104-109 Gy/min). The front (anterior) of the phantom was oriented closest to the South elements for all exposures. The unilateral exposure used only the South source elements. Each alanine vial contained 3 pellets. The dosimetry vials were placed in sample opens made for them, which are directly behind and adjacent to the positions for the biological sample vials. For scattering consistency empty sample vials were in each of those 12 sample positions. Likewise empty dosimetry vials were included in each experimental exposure using the virus samples. An e-scan Alanine Dosimeter Reader (Bruker BioSpin, Corp., Billerica, MA) EPR instrument was used to determine the dose absorbed by the dosimetry pellets. The resulting dose rate maps are presented in Figure 1. For all experiments great care was taken to place the phantom in exactly the same position relative to the sources for all exposures, dosimetry or experimental, regardless of the configuration or covering materials.

Virus Gamma Irradiation

Vaccinia virus was exposed to gamma radiation in the Armed Forces Radiobiology Research Institute (Bethesda, MD) cobalt-60 facility. Prior to irradiation, the virus was thawed on ice for 30 minutes then equilibrated to ambient temperature for 15 minutes. The virus was exposed to 30kGy of gamma photons under various apparatus scenarios; for each scenario 12 samples of virus were equally divided among 4 transverse slices of a human phantom model (Figures 2-2a and 2-2b). The first irradiation scenario was gamma exposure for the human phantom covered in a government spec human remains pouch (Figure 2-3). The second scenario was virus in the human phantom that was dressed with an Improved Outer Tactical Vest that contained forward and rear Enhanced Small Arms Protective Inserts inside the human remains pouch (Figures 2-4a, 2-4b, and 2-4c), all enclosed within the aluminum case (Figures 2-5a and 2-5b). Post-irradiation, the viral samples were returned to ice for 15 minutes before storage at -80°C. Exposure of virus for the other studies was done in a previously designed and extensively used and characterized experimental set up. Briefly, it is a rectangular water bath with sample positions for up to 40 samples. Each sample vial is contained within a 15 ml test tube as a secondary container. Other handling and dosimetry is as described.

Plaque assays

BS-C-1 cells (ATCC #CCL-26) were seeded onto 6-well tissue culture plates 18-24 hours prior to assay at a density of 5×10^5 cells per well in growth medium (Minimal Essential Medium with Earle's Balanced Salt Solution, 10% non-heat inactivated FBS, 2mM L-glutamine, 50µg/mL gentamicin). All incubations for this cell type and assay were at 37°C, 5% CO₂. A 1:1 mixture was made with a 200µl vaccinia sample and 0.25mg/mL trypsin from porcine pancreas (Sigma, Inc.). If the vaccinia was previously dried, it was reconstituted in 200µl diH₂O just prior to assaying. This virus/trypsin mixture was incubated at 37°C for 30 minutes, alternating between vortexing for 10 seconds and sonicating for 30 seconds every 5 minutes. The virus/trypsin was then titrated in MEM-2.5. After washing the BS-C-1 cells twice with PBS, they are infected with 500µl of the various titrations of the virus/trypsin/media mixture for 2 hours, rocking every 30 minutes. Following infection, 2mL of methylcellulose medium (0.5% methylcellulose, 5% non-heat inactivated FBS, 1mM L-glutamine) was added to the wells for a 72 hour incubation. To stain the plaques, the methylcellulose medium and virus were aspirated out of the wells and the cells were washed twice with PBS. A 0.1% crystal violet solution in 20% ethanol was applied to the cells for 5 minutes. The stain was then discarded and the wells of the plate allowed to air dry for proper visualization and titer determination.

Amplification of Vaccinia Virus WR and Lister

Crude hydrated vaccinia virus was incubated at 37°C for 30 minutes, alternating between vortexing for 10 seconds and sonicating for 30 seconds every 5 minutes. Cellular debris was centrifuged down at 500 x g for 5

minutes. The supernatant was collected for DNA extraction using phenol:chloroform. PCR was performed in a total volume of 50µl using a TITANIUM *Taq* PCR Kit (Clontech Laboratories, Inc.), 20ng of extracted DNA, 5pmol each of custom forward and reverse primers (Integrated DNA Technologies, Inc.), and 5% DMSO. The sequences for these primers are 5' AATACAAGGAGGATCT 3' and 5' CTTAACTTTTCTTTCTC 3'. After initial denaturation at 94°C for 10 minutes, 35 cycles of amplification was performed; this included a denaturation step for 1 minute at 94°C, an annealing step at either 40.6°C (WR) or 46.5°C (Lister), and an elongation step at 72°C for 2.5 minutes. Following the cycles, a final elongation step at 72°C was extended to 10 minutes to ensure complete extension of DNA products. The PCR products were analyzed on a 1% agarose gel.

XbaI digestion of amplicons

NEBuffer 4 and 30 units of *XbaI* (New England Biolabs, Inc.) were added to 3µg PCR product and brought up to a total volume of 15µl. This mixture was incubated at 37°C for 2 hours, and digestion products were analyzed on a 2% agarose gel.

Appendix 2

Photos of the Human Phantom and Associated Equipment

Figure 2-1



Figure 2-1 The top vial holds alanine pellets for dosimetry purposes. The bottom vial is an o-ring sealed cryovial that was heat-sealed. The two areas to the left are the holes in the phantom for the vials. The white objects are indexing points and part of the phantom construction.

Figure 2-2A and 2-2B

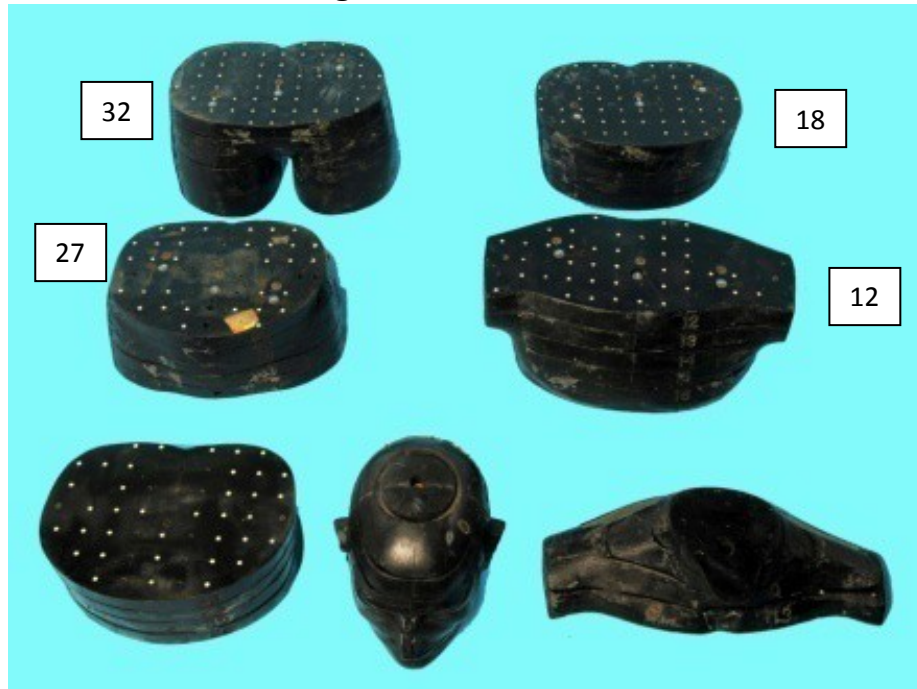


Figure 2-2A The human phantom is comprised of multiple transverse slices (numbered 0-35 from the head (0), lowest to torso piece (32). Slices with vials are labeled. Twelve virus samples are divided evenly between slices 12, 18, 27, and 32. Assembly is head, right most 3 sets of slices then left most sets of slices.



Figure 2-2B For each cobalt-60 exposure, dosimetry vials and sample vials are present. For dosimetry exposures the dosimetry vials contain alanine pellets and the sample vials are empty. For exposures with virus the dosimetry vials are empty

Figure 2-3



Figure 2-3 For the first irradiation scenario using virus the human phantom was exposed while inside a government spec human remains pouch.

Figure 2-4A, 2-4B, 2-4C

For the second irradiation scenario, the human phantom containing viral samples was exposed while wearing an IOTV inside the remains pouch and aluminum case (Figures 2-4 and 2-5 below).

Figure 2- 4A. To the right of the human phantom are ESAPI plates with one each placed in the front and back of the IOTV.





Figure 2- 4B (left panel) .The human phantom wearing the IOTV with ESAPI plates.

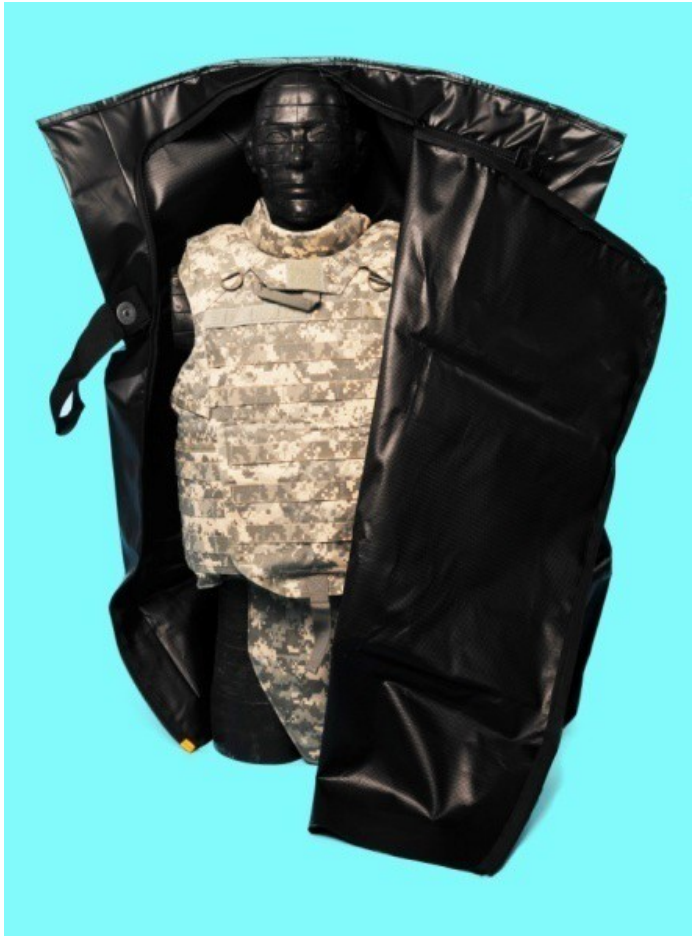


Figure 2- 4C (right panel). The phantom was then placed inside the government specification human remains pouch for irradiation.

Figure 2-5A and 2-5B

An aluminum case slightly larger than the human phantom was custom fabricated. The phantom containing virus samples was wearing an IOTV with ESAPI plates, and placed inside both the government spec human remains pouch and the aluminum case. Note that the case has a separate aluminum plate on the bottom so that the box can be open towards that end, allowing for the entire case to slide over the human phantom. The phantom is shown without the IOTV and pouch for scaling purposes (Appendix 3).

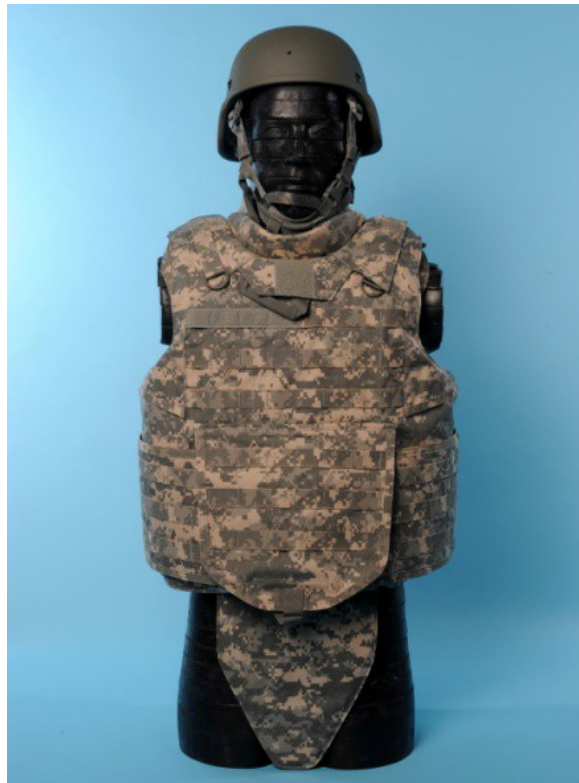
Figure 5A

Figure 5B



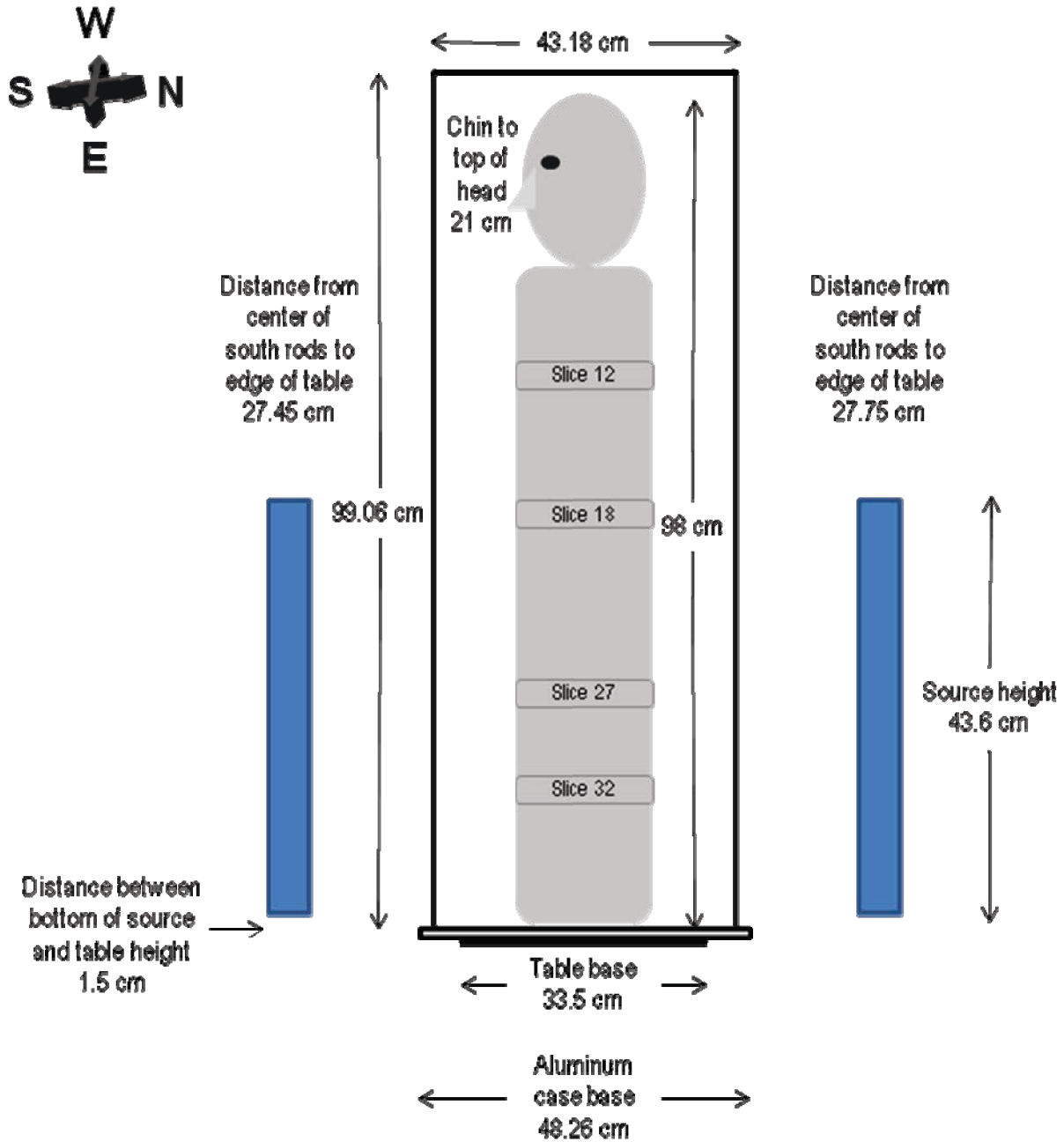
Figure 2-6A, 2-6B, 2-6C

Dosimetry analysis was conducted to determine if an ACH would attenuate radiation effects. Dosimetry analysis was conducted with a vial containing alanine pellets located in the head portion of the phantom (slice 1; single vial position). In addition to the ACH, the IOTV and ESAPI plates were also on the phantom. For this exposure no viral samples were present nor processed

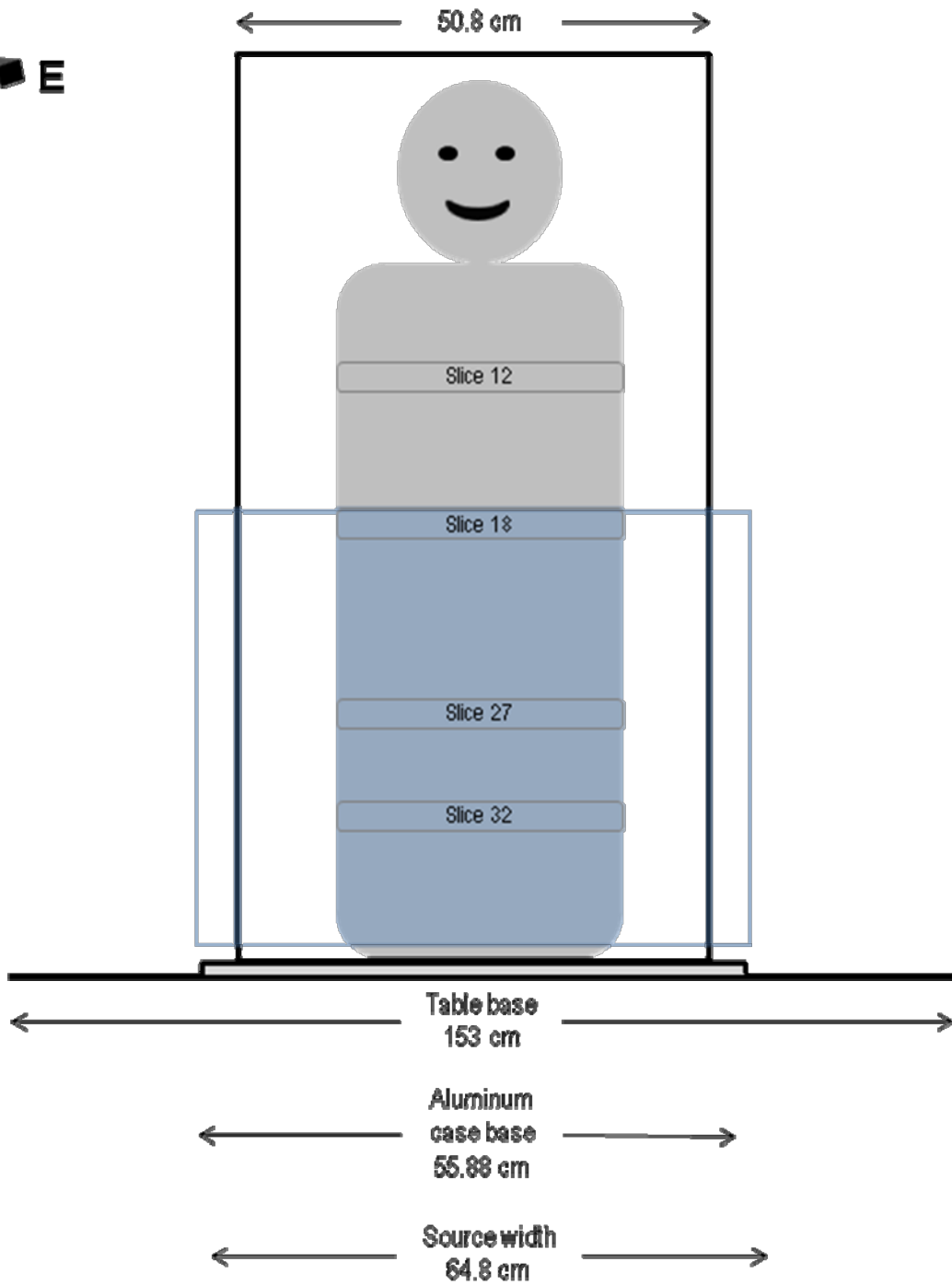


Appendix 3

Human Phantom and Radiation Source Diagram



Slice 12: 17 cm
Slice 18: 23 cm
Slice 27: 21.5 cm
Slice 32: 22 cm



Slice 12: 39.5 cm
Slice 18: 31.5 cm
Slice 27: 28 cm
Slice 32: 32 cm

Appendix 4

Virus Titer Data Summary – Phantom Exposed Samples - Phantom with Body Pouch

Sample #	Exposure Date	Virus Lot	Original Titer	Strain	Experimental Set Up	Date Titered	Target Dose (kGy)	Avg Dose Rate to Water (kGy/h)	Exposure Time (h:mm:ss)	Actual Dose (Avg, kGy)	Positional Dose Rate	Positional Total Dose	Temp (°C)	Beginning Temp (°C)	End Temp (°C)	Titer
1108	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/1/10	30	4.59	6:32:34	29.94	4.88	31.73	RT	18	33.3	0.00E+00
1109	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/8/10	30	4.59	6:32:34	29.94	4.67	30.35	RT	18	33.3	0.00E+00
1110	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/18/10	30	4.59	6:32:34	29.94	4.85	31.54	RT	18	33.3	0.00E+00
1111	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/1/10	30	4.59	6:32:34	29.94	6.19	40.22	RT	18	33.3	0.00E+00
1112	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/8/10	30	4.59	6:32:34	29.94	5.73	37.26	RT	18	33.3	0.00E+00
1113	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/18/10	30	4.59	6:32:34	29.94	6.19	40.25	RT	18	33.3	0.00E+00
1114	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/1/10	30	4.59	6:32:34	29.94	5.87	38.15	RT	18	33.3	0.00E+00
1115	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/8/10	30	4.59	6:32:34	29.94	5.76	37.43	RT	18	33.3	0.00E+00
1116	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/18/10	30	4.59	6:32:34	29.94	5.66	36.80	RT	18	33.3	0.00E+00
1117	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/1/10	30	4.59	6:32:34	29.94	4.77	30.99	RT	18	33.3	0.00E+00
1118	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/8/10	30	4.59	6:32:34	29.94	4.76	30.95	RT	18	33.3	0.00E+00
1119	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/18/10	30	4.59	6:32:34	29.94	5.15	33.50	RT	18	33.3	0.00E+00
1120	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/4/10	0	0	NA	0	0	0	RT	22	22	2.44E+09
1121	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/22/10	0	0	NA	0	0	0	RT	22	22	2.60E+09
1122	9/23/10	8/20/10 JG	3.12E+09	Lister CD	Phantom, pouch	10/22/10	0	0	NA	0	0	0	RT	22	22	3.08E+09

Virus titer data for all samples exposed inside the phantom and the unirradiated matched controls. As expected all radiation exposed sample were completely inactivated. All samples were from the same preparative lot 8/20/10 JG. The human phantom faces south wall of Cobalt Facility. Samples placed in ascending order from top to bottom, west to east e.g. the first sample, 1108, was placed in the west (left most when facing the phantom) most position in slice 18 the last 1122 in the east/right most position of slice 32. Note there is a slight decrease in titer for the unirradiated controls due from the initial titer, due to experimental

handling, but the titer remains very high. Virus strain is Lister and the preparation is crude dry (CD). Original titer was determined for the virus immediately post-growth. The free in air total dose, e.g. external total dose was 42.3 kGy based on a dose rate of 6.468 kGy/h.

Virus Titer Data Summary – Phantom Exposed Samples – Phantom with Body Armor, Body Pouch, and Inside Case

Sam p. #	Exposure Date	Original Titer	Strain	Experimental Set Up	Date Titered	Target Dose (kGy)	Avg Dose Rate to Water (kGy/h)	Exposure Times (h:mm:ss)	Avg. Dose (kGy)	Avg. Dose Total (kGy)	Position. Dose Rate	Position Dose - 1st Fraction	Position Dose - 2nd Fraction	Total Posit. Dose (kGy)	Initial Temp (°C)	End Temp (°C)	Titer
1123	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/5/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.136	16.54	16.83	33.38	22	29	0.00E+00
1124	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	3.771	15.08	15.35	30.43	22	29	0.00E+00
1125	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.015	16.06	16.34	32.40	22	29	0.00E+00
1126	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/5/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	5.333	21.33	21.71	43.04	22	29	0.00E+00
1127	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.754	19.02	19.35	38.36	22	29	0.00E+00
1128	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	5.211	20.84	21.21	42.05	22	29	0.00E+00
1129	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/5/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	5.265	21.06	21.43	42.49	22	29	0.00E+00
1130	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	5.139	20.56	20.92	41.47	22	29	0.00E+00
1131	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	5.051	20.20	20.56	40.76	22	29	0.00E+00
1132	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/5/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.551	18.20	18.52	36.73	22	29	0.00E+00
1133	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.506	18.02	18.34	36.36	22	29	0.00E+00
1134	10/26 & 27 2010	3.12E+09	Lister CD	Phan, vest, pouch, case	11/11/10	30	3.717	4:00:01, 4:04:17	14.87, 15.13	30.00	4.817	19.27	19.61	38.87	22	29	0.00E+00
1135	10/26 & 27 2010 (-)	3.12E+09	Lister CD	Phan, vest, pouch, case	11/8/10	0	NA			0				0	22	22	2.14E+09
1136	10/26 & 27 2010 (-)	3.12E+09	Lister CD	Phan, vest, pouch, case	11/19/10	0	NA			0				0	22	22	2.80E+09
1137	10/26 & 27 2010 (-)	3.12E+09	Lister CD	Phan, vest, pouch, case	11/19/10	0	NA			0				0	22	22	1.70E+09

Virus titer data for all samples exposed inside the phantom, covered with the pouch, vest and case, and the unirradiated matched controls. As expected all radiation exposed sample were completely inactivated. All samples were from the same preparative lot 8/20/10 JG. Human phantom faces south wall of Cobalt Facility. Samples placed in ascending order from top to bottom,

west to east. Exposures were fractioned using two nearly identical exposure periods on two successive days. Average doses based on the average dose rate and the individual doses based on the dosimetry at each sample position were calculated. The free in air total dose, e.g. external total dose was 51.7 kGy based on a dose rate of 6.402 kGy/h and total exposure time.

Appendix 5

Virus Titer Data Summary –Water Bath Exposed Samples – Effects of High Potassium

Sample #	Exposure Date	Original Titer	Strain	Comments	Date Titered	Target Dose (kGy)	Exposure Time (h:mm:ss)	Avg Dose Rate to Water (kGy/h)	Actual Dose (Avg, kGy)	Temp (°C)	Beginning Temp (°C)	End Temp (°C)	Titer
1002	5/26/10	6.80E+09	WR CH		7/2/2010	0		6.53	0	RT			3.60E+09
1004	5/26/10	6.80E+09	WR CH		6/28/10	10	1:38	6.53	9.995	RT			1.80E+07
1005	5/26/10	6.80E+09	WR CH		7/2/2010	10	1:38	6.53	9.995	RT			7.10E+06
1007	5/26/10	6.80E+09	WR CH	~150mM K+	6/28/10	0		6.53		RT			2.80E+09
1008	5/26/10	6.80E+09	WR CH	~150mM K+	7/2/10	0		6.53		RT			2.12E+09
1010	5/26/10	6.80E+09	WR CH	~150mM K+	6/28/10	10	1:38	6.53	9.995	RT			2.00E+07
1011	5/26/10	6.80E+09	WR CH	~150mM K+	7/2/10	10	1:38	6.53	9.995	RT			3.60E+07
1002	5/26/10	6.80E+09	WR CH		7/2/2010	0		6.53		RT			3.60E+09
1004	5/26/10	6.80E+09	WR CH		6/28/10	10	1:38	6.53	9.995	RT			1.80E+07
1005	5/26/10	6.80E+09	WR CH		7/2/2010	10	1:38	6.53	9.995	RT			7.10E+06
1007	5/26/10	6.80E+09	WR CH	~150mM K+	6/28/10	0		6.53		RT			2.80E+09
1008	5/26/10	6.80E+09	WR CH	~150mM K+	7/2/10	0		6.53		RT			2.12E+09
1010	5/26/10	6.80E+09	WR CH	~150mM K+	6/28/10	10	1:38	6.53	9.995	RT			2.00E+07
1011	5/26/10	6.80E+09	WR CH	~150mM K+	7/2/10	10	1:38	6.53	9.995	RT			3.60E+07

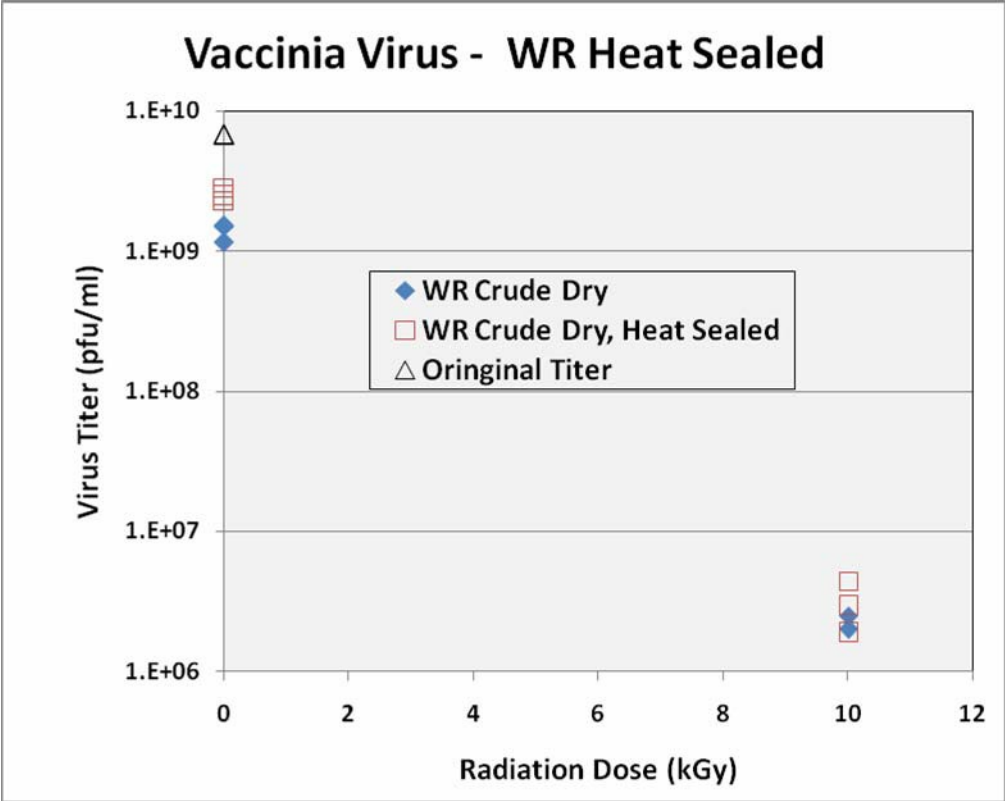
Samples having normal or high potassium content (150 mM). All samples from virus lot 4/19/10 JG. Exposures were done using a water bath array holding up to 40 samples. Samples are contained within a 1.5 ml microcentrifuge tube placed inside a 15 ml centrifuge tube. Virus samples were the WR strain and were suspensions of the virus and lysed cultured cells used to grow the virus. The potassium concentration of some samples was elevated by adding a concentrated stock solution. Original titer was determined for the virus immediately post-growth.

Appendix 6

Virus Titer Data Summary — Effects of Heat Sealing Samples

Standard safety practice for handling even biosafety level 2 infectious disease agents is to have them contained in a primary and secondary outer container. The sample holes within the human phantom were just large enough to hold the 1.5 ml cyotubes. Enlargement of the holes would have made the dose received to the sample less accurate. As an alternate the heat shrinkable tubing material to seal tubes for cryopreservation were used to seal the tubes. This increased the overall physical integrity and prevented loosening of the screw cap. A concern was although a rapid and brief heat exposure was used to shrink the tubing, it would affect the virus titer. Data in the Table and Figure show that there was no effect.

Sample #	Exposure Date	Virus Lot	Original Titer	Strain	Comments	Date Titered	Target Dose (kGy)	Exposure Time (h:mm:ss)	Actual Dose (Avg, kGy)	Avg Dose Rate to Water (kGy/h)	Temp (°C)	Titer
1012	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/12/10 PSW	0	0	0	6.53	RT	1.50E+09
1013	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/12/10 PSW	0	0	0	6.53	RT	1.52E+09
1014	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/23/10 PSW	0	0	0	6.53	RT	1.16E+09
1015	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/12/10 JG	10	1:32:48	9.995	6.53	RT	8.00E+05
1016	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/23/10 JG	10	1:32:48	9.995	6.53	RT	2.48E+06
1017	5/26/10	4/19/10 JG	6.80E+09	WR CD		7/23/10 JG	10	1:32:48	9.995	6.53	RT	2.00E+06
1018	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/16/10 JG	0	0	0	6.53	RT	2.82E+09
1019	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/30/10 JG	0	0	0	6.53	RT	2.28E+09
1020	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/30/10 JG	0	0	0	6.53	RT	2.46E+09
1021	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/16/10 JG	10	1:32:48	9.995	6.53	RT	4.40E+06
1022	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/26/10 JG	10	1:32:48	9.995	6.53	RT	1.88E+06
1023	5/26/10	4/19/10 JG	6.80E+09	WR CD	Heat seal	7/26/10 JG	10	1:32:48	9.995	6.53	RT	2.92E+06



The data show that heating (red squares) alone did not reduce the titer relative to matched handling controls (blue diamond). Furthermore there was no change in the radiation sensitivity based on the 10 kGy exposure. A small reduction in titer from that measured (black diamond) when first harvested due to routine experimental handling.

**Contaminated Human Remains: Transportable Decontamination:
Current State of Technology Relevant to Development of a Transportable System
for Treatment of Contaminated Human Remains**

Project Irradiation Technology “White Paper” Report

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Project Number: CA09DEC015
Capability Area: Biological Agent Neutralization

Introduction

The goal of this document is to summarize additional information applicable to the use of ionizing radiation for the decontamination of human remains.

The inability to safely return biologically contaminated remains to the families of service members who died in defense of their country has been a persistent problem. A number of approaches have been considered, but all have problems and short falls. These concerns have been reviewed and documented in "Initial Capabilities Document for Mortuary Affairs Operations, V6.7", October 2008.

Use of ionizing radiation provides a means of sterilization for which there can be the highest confidence that no viable disease causing organisms survive and minimize risks to mortuary personnel and the environment. Due to the high penetration through biological materials biological agents would be killed even in interior body areas, such as the lungs, abdominal cavity, and gastrointestinal track. No surgical opening or penetration (trochors) would be necessary to introduce materials into these tissue cavities, therefore reducing the possibility of aerosols and minimizing hazards to personnel and the environment. Also sterilization is not dependent on an intact vascular system, and therefore the process is applicable to degraded remains as well. Once treated remains would not be subject to further decomposition by bacterial action, as both spore and vegetative forms would be killed. As in mass casualty situations refrigeration is limited and there are considerable potential health hazards to handling remains (Morgan et al. 2006; ICDMAO V6.7, 2008) this could be an important advantage in using ionizing radiation sterilization.

Sterilization can be accomplished for remains which are already contained, either in light materials (natural or plastic) such as bags, or in heavier materials, such as metal containers including Zeigler cases. The technology for construction of devices that produce ionizing radiation is well established. Industrial and medical irradiators are commonly based on devices that do not use radioisotopes, and therefore minimize the hazard or security concerns. No or minimal use of water is necessary and no chemical agents are required to be transported or disposed of after use. There are now source technologies that are robust enough that they can be engineered to operate in field environments.

As detailed below the other technical and administrative procedures for determining an effective process and approving and monitoring process exists within the industrial and medical irradiation industry and similar approaches could be readily applied to for development, approval and fielding of this specialized application.

Previous Work

DoD Mortuary Community Interactions

During the period of approximately 2002 to 2004 AFRRRI scientists had extensive conversations and presented several briefing to members of the military mortuary community including the Mortuary Affairs and Casualty Support Division, Casualty and Memorial Affairs Operations Center, US Army Human Resources Command and DoD and Health Affairs staff members. The appendix to this

document reproduces the summary findings from those meetings; while the next section summarizes the research activity and data.

Equipment Design Concept

Pursuant to the information received from the Central Joint Mortuary Affairs and Operations (CJMAO) the following device concept was developed at that time, consisting of several components and activities. These concepts had their basis in technical discussion held with other government and private sector scientists and engineers for other agent defeat projects including those addressing the attacks on the US Postal Service.

A high energy electron source would be used to produce X-rays. The source and its power supply were scaled to produce X-rays of sufficient energy to readily penetrate human remains, including those in Kelvar body armor and/or encased in aluminum Zeigler cases. The size and weight of the source and electrical power generator were constrained by transport needs. The weight and size proposed were restricted to be consistent with the source and power supply each being able to be transported by air on standard sized pallets and on the ground by a 2.5 ton truck ("deuce and a half") or similar transport vehicle.

Estimates were made of the source characteristics based on the available commercial off the shelf high energy electron sources available at that time and treatment rate estimates provided. The trade off is higher treatment rates require higher dose rates which require equipment which is comparatively larger. Those which could meet the size and weight requirements for transportability ranged in power levels were estimated to be between 3 kW and 1000 kW. Taking into account dose rates it was estimated the number of remains that could be treated per hour ranged from 3 to 20 individual remains from the lowest to highest source power levels. This was based on information about the relationship between power, X-ray conversion, resulting dose rate, and at total required dose of 120 kGy. This total dose was based on the least sensitive biological agents being bacterial spores and viruses, the radiation sensitivity data available and an estimated 12 log reduction in viability being necessary. More recent work on target agent radiation sensitivity (see below) suggests this maybe higher than necessary.

A field site was anticipated to consist of the source, its power supply, shielding and standard mortuary affairs activities. The source design was to point down ward into a trench, which would provide radiation shielding to operators, mortuary, and support personnel. Therefore equipment to dig a trench and/or form beams of approximately 6 feet deep/high and 10-30 feet long would be needed. It was expected the site would include the other mortuary affairs operations for remains identification, securing of personnel effects and record keeping as well as pre-treatment and post-treatment staging areas.

No final determination was made as to how remains would be translated through the source radiation field. Options which were considered were manual placement, use of semi-manual methods, such as the roller systems already in use for remains handling, or fully automatic powered conveyor systems. A field ready system could incorporate all of these systems, with appropriate standard operating procedures, and therefore it was possible that all these methods could be included for maximum field operational flexibility.

Proof of Principle Experiment

As a proof of principle an initial scoping experiment AFRRRI scientists was conducted during this period. The results were provided to the CJMAO, and was submitted for review to the by the Armed Forces Epidemiology Board CDC mortuary affairs board.

The experiments conducted used dry bacterial spores, *Bacillus anthrphaeus* (previously *B. subtilis* var, niger or *B. globigii*). Twelve vials of spores containing 0.5 grams and 12 vials containing alanine pellets for dosimetry were placed through out the volume of a human phantom. The human phantom was then covered with both typical body amour and a uniform and placed in a 16 gage aluminum case. This configuration tested the ability of ionizing radiation to inactivate bacterial spores despite shielding from typical military garments, over pack and the differences in human body torso density, all of which could change the actual radiation dose delivered to specific points within the human torso model. The radiation source used was a form of electron accelerator (Rhodotron) with conversion of the high energy electrons to X-rays. The measured dose through out the torso ranged from 45 to 53 kGy. The initial spore concentration was 5×10^{11} colony forming units (CFU) per gram. The spore viability was evaluated by standard plating methods for all of the vials. No viable spores were recovered at any location for a growth period of 12 days.

Evaluation of Early Concept

This proof of concept experiment was reviewed by the Armed Forces Epidemiology Board who issued a letter (see Appendix). Among their conclusions was that use of X-ray radiation was an effective means of sanitizing human remains contaminated with spore or vegetative *Bacillus anthraces* bacteria.

Based on these findings it was concluded by CJMAO that a transportable system was a viable approach to meet their needs and AFRRRI scientists were requested to provide technical support for acquisition. The acquisition process was begun. However, subsequent loss of funding for new technology in this area stopped further pursuit of acquiring the equipment either as a full scale test bed or field ready system at that time.

Application of Ionizing Radiation to Human Remains

Applying the well established technology of ionizing radiation disinfection to the specific application of sterilization of human remains involves revolving questions in the following areas.

- First is establishing the specific total ionizing radiation dose necessary.
- Second is defining the operational needs based on the requirements of the mortuary community.
- Third is establishing the type of radiation source and specific engineering design to meet both the treatment efficacy and deployment requirements of a usable real world device.

- Lastly is to identify the regulatory authorities for the development and approval of this application and those responsible for the later oversight and regulation during its actual use.

All of these aspects are those routinely addressed during the application of ionizing radiation sterilization technology in an industrial setting, either when there is a new user of existing applications or there is a new application for radiation based processing.

The most recent novel application of radiation based disinfection is the treatment of the US Mail prior to delivery. The methodology was established relative quickly and implemented over the course of several months. The participation agencies in this effort included the US Postal Service, the Armed Forces Radiobiology Research Institute, National Institute of Standards and Technology, and the Food and Drug Administration, all in collaboration with the Office of the Scientific Advisor to the President.

The subsequent sections will provide details on the issues and the state of the art or based on previous experience and knowledge about the technical or administrative issues that likely will need to be addressed. At the end of this document a brief primary on concepts and background concerning ionizing radiation decontamination is provided; and for additional information the literature cited includes major reference works on disinfection and sterilization.

Total Dose of Radiation Needed for Biological Threat Agents

The total radiation dose to be used is determined by the level of contamination (bio-burden), the safety assurance level desired, and the radiation sensitivity of the microorganisms present.

Good practice for all disinfection process is to use treatment conditions that reduce the bio-burden below the one viable organism per gram (or milliliter) to levels in which there would be one organism per kilograms or greater amounts of material. This target level is the Safety Assurance Level (the SAL) and is general set based on the virulence of the microbial pathogen, the number of organisms necessary to cause infection, the Estimated Infectious Dose (EID), the availability of treatments, and the amount of material one could be exposed to that could contain one EID. The SAL is generally set so it is unlikely that anyone exposed to the decontaminated material could reasonably be exposed to an EID. In food and industrial sterilization the SAL rule of thumb has been generally a 6 log reduction, assuming a bio-burden of 1×10^6 viable microbes, or a 12 log total reduction in viability.

The microbial pathogens of concern for human remains are likely to be at high levels of bio-burden and be of great concern as there maybe no medical treatment available and/or are highly virulent. The levels of contamination potentially will reach the maximum that bacteria, bacterial spores and viruses can obtain in culture or even higher externally, if significant concentration of materials is done prior to their release. Concentrations of bacteria, bacterial spores and viruses could readily reach concentrations requiring log reductions of 10-12 logs just to reach levels that are undetectable by standard biological assay methods. Greater reduction will likely be considered necessary, resulting in the SAL being set such that more than 12 logs of reduction are necessary.

Note these issues and concepts are not unique to radiation sanitization of human remains, but apply to all sanitization or sterilization methods.

As there is an extensive literature, on microbial radiation sensitivity it would be possible to set an upper limit for radiation dose, once a safety assurance level was established by the medical and mortuary affairs communities. The most conservative total dose would be based on using the upper limit of D_{10} values for both bacterial spores and viruses. The actual value should be agreed on by a group of experts on radiation based sterilization and/or threat agent characteristics.

Currently D_{10} values are available for many major potential threat agents or the only available stimulants that can be used. Examples are as follows:

- *Bacillus anthracis* spores 1.0 to 5.7 kGy
- Vaccinia virus (stimulant for smallpox virus; *Varola major*) 1.0 to 9.9 kGy
- Ebola virus 1.4 to 2.2 kGy
- Lassa Fever Virus 1.9 to 3.1 kGy
- Marbug Virus 1.2 to 2.1 kGy
- Influenza A Virus 2.5-7.1 kGy
- Ricin Toxin 70 kGy

The values above are from the open literature and are for gamma photon radiation exposures only. Ranges are given, as these reflect variations observed reflecting both biological and radiation exposure variables used in the specific studies. For radiation based sterilization values for X-rays and gamma photons are considered to first approximation similar. The references in the bibliography provide details on the specific conditions and characteristics. The appendix and more general references provide general principles of radiation sterilization.

Using these values and assuming a bio-burden of 10^{12} and a SAL of 3 logs then 15 logs of dose would be necessary requiring between 15 and 150 kGy doses of radiation for bacterial and virus decontamination. This highest dose is based on the highest D_{10} values observed for viruses and is a “worst case” upper limit. Vegetative bacteria, which are the primary cause of decomposition, usually have radiation sensitivities much lower than possible threat agents. As discussed below exposure conditions or other factors could lower this value considerably.

Therefore the remains could be treated so that they would be sterile, subject to no further decomposition and no longer be a medical threat.

These radiation sensitivity values available from the open literature studies provide a great deal of generally applicable data. However the conditions of exposure and the specific agents used are primarily those of concern for food and medical devices. Much of the available data does not

address the radiation sensitivity of possible threat agents or a broader set of radiation exposure conditions or physical and chemical conditions, temperature, and wetness or dryness, that might be encountered the field environments where inactivation of microbial agents might need to be done for decontamination of remains. .

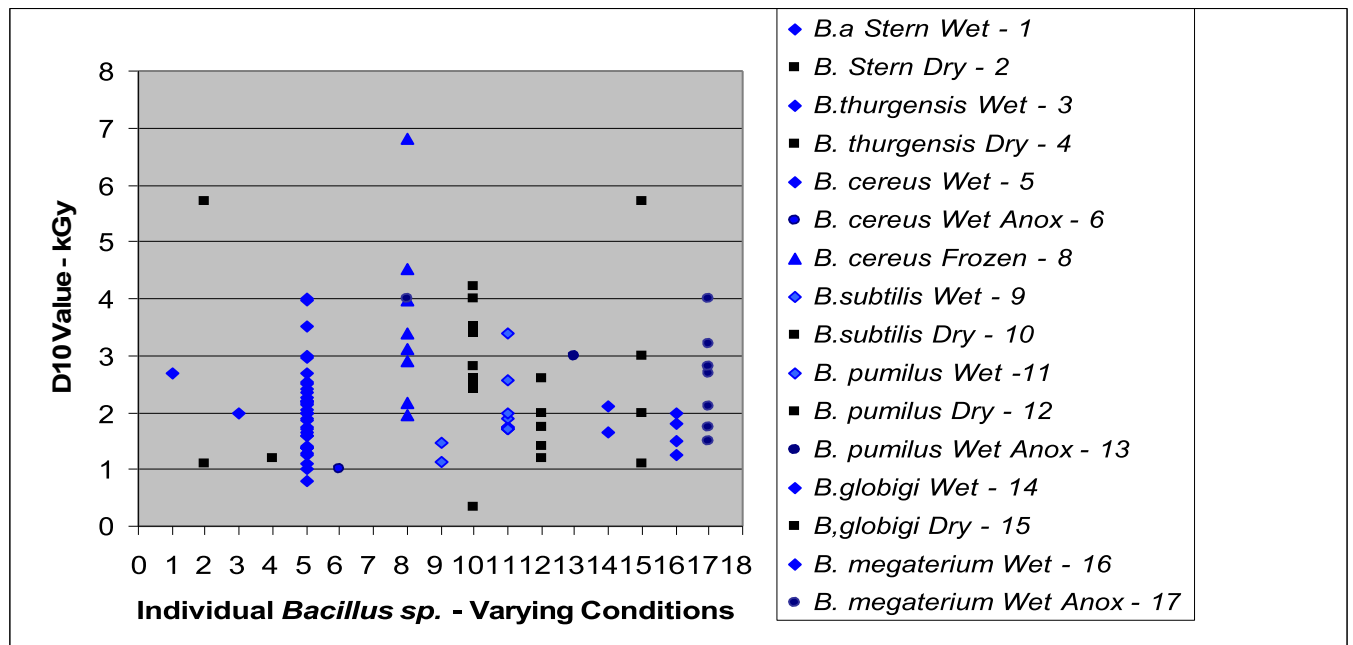
Therefore periodically DoD has funded research on microbial radiation sensitivity. Both from older studies and more recent experimental work there is now additional information on possible threat agent radiation sensitivity for many but not all the potentially relevant physical-chemical conditions or for the entire radiation source. Some of this additional information on specific values radiation sensitivity values are currently available in documents with limited, need to know circulation. Primary sources for these publications are the DITIC and STARS (DTRA) data bases and the documents associated with the USAF Empirical Lethality Method program. Taking these additional available data into account potentially will lower the total radiation dose necessary. However this is highly dependent on information from the mortuary affairs community.

Use of Specifically Determined Radiation Sensitivity Values

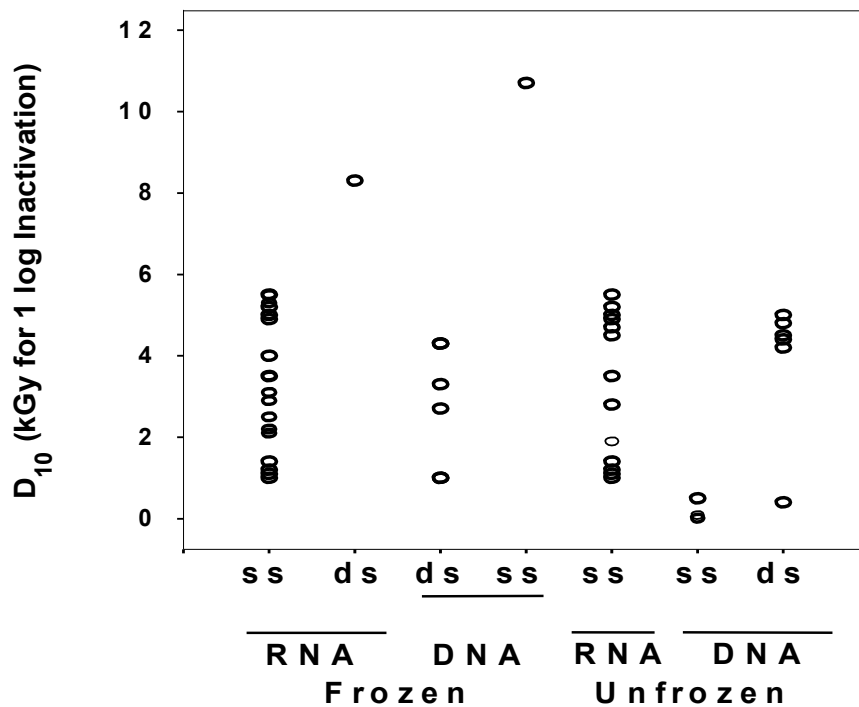
- It is necessary to establish the D_{10} value specifically applicable to the organisms, the physical chemical conditions and radiation source characteristics to obtain the maximize efficiency of the sanitization or sterilization process.
- Additional regulatory and certification may require use of such specifically applicable D_{10} values for maximum assurance of efficacy for the radiation sterilization the process and its approval. .

As the D_{10} value is the slope of the inactivation curve, what appear to be small changes in values can have large impact on processing efficiency and the total dose necessary to reach the SAL.

The following two graphs illustrate the variability of D_{10} values that exists from open literature publications. The first figure is for *Bacillus* spores. Included are multiple species and exposures done on dry, hydrated and frozen spores and with and without oxygen present (anox). The second figure shows the D_{10} for viruses sorted by genome structure, and whether exposed in the frozen or liquid hydrates state. The variation in D_{10} values reflects all of these physical factors, as well as that the studies were done by different researchers, using slightly different evaluation methods and radiation exposure conditions.



Data from Russell, 1982



Graph is from Lowy, 2003. Data are for multiple types of viruses exposed to ionizing radiation. Reported values were sorted by type of genome and whether exposures were done in liquid media or frozen. Genome types are ss (single strand) ds (double strand) and DNA and RNA and included major groups and types of viral pathogens.

The most important point from these data is that reported range of D_{10} values can be broad for either broad classes or even a relatively specific group of microbial agents.

The conditions that occur for remains that are likely to affect radiation sensitivity are the target organisms, the hydration state and the temperature during the radiation process. It is also possible the pH of the tissues and their state of decomposition could alter radiation sensitivity. Based on the range of D_{10} values observed for similar biological and physical-chemical affects it is expected that D_{10} values for a given target microbe can be altered generally by a factor of 2-3 fold. But use of the most applicable D_{10} value provides the information necessary so that the minimum time can be used to reach the desired total dose to obtain SAL.

Example: Consider a virus for which a D_{10} value of 5 kGy was determined under one set of conditions. If the log reduction is required for sterilization and safety is determined to be 11 logs then ~55 kGy of total dose will be necessary. But if it can be shown that the due to the virus being in a different exposure environment, where the D_{10} decreases to 2 kGy then 22 kGy of total dose will be needed. If the radiation source is capable of producing 10 kGy per hour then processing will take either 5.5 hours or 2.2 hours for the same amount of material. **Therefore in a 24 hour period 2.5 times more processing can be accomplished if the lower D_{10} value is applicable.**

Effect of Ionizing Radiation on Remains

There have been few specific studies to examine the effects of high dose ionizing radiation on human tissues after death. Radiation damage to skin due to accidents, radiotherapy and nuclear weapon detonations is well known, but is primarily the result of complex physiological responses by the living dermis (Nias 1998; Walker and Cerveny 1989). There are reports for radiation treatment ex vivo of both soft tissues, including skin, and hard tissues, bone, for the purposed of tissue banking and allogenic transplantation. Recent reports showed that there was little change in either skin or bone properties for doses of 50 kGy of ionizing radiation (Grieba et al. 2005; Rooney et al. 2008; references there in). It was also shown these doses were capable of inactivating up to 10^9 bacterial spores and a number of other viral and bacterial pathogens. In these reports the tissues were treated with glycerol, a radioprotectant, which would be expected to minimize damage to the tissues and make microbial killing more difficult. Therefore these studies confirm the ability of ionizing to inactivate pathogens in human tissues. The levels of joule heating due to radiation energy absorption are likely low enough that would not be sufficient to cause appearance changes or burning. Whether there would be other dermatological effects or changes in human remains by a radiation treatment process remains to be determined.

Irradiation Devices and Facilities

An irradiation facility broadly consists of three components: a) the radiation source including it's controls and shielding b) the mechanism for safely moving material to be exposed into the radiation field and c) the support facilities, such as material storage pre- and post treatment and d) power supply. Commercial irradiation facilities use sources based on radioisotopes, cobalt-60 or cesium-137, or the production of high energy electrons with or without conversion to X-rays.

Previous discussions with the mortuary community emphasized that the best solution is transportable or portable equipment. The type of radiation source at this time most likely to successfully address that need is based on devices producing high energy electrons (HeV), which are accelerator technologies or possibly X-ray machines. High energy electron sources alone are unlikely to be useful, due to the lack of penetration by HeV. Generally doses can be delivered

through a thickness of 4 cm of water and slightly less for tissue equivalent material. However HeV sources can produce X-rays at high dose rates, with large field sizes. High energy X-rays have high penetration through both light density, biological materials, and through denser materials, metals. Illustrative is the use of high energy x-rays to inspect structures and metal fabrications for internal flaws.

Isotope based facilities are less likely to be an attractive technology for a transportable device. Safe transport of these materials having sufficient activity to provide the necessary dose rates require both heavy shielding and special containers to avoid release of the isotope materials. Movement of these materials generally requires extensive prior notification of appropriate regulatory agencies. Heavy shielding within the irradiation facility is necessary to protect operators and bystander personnel. Also there are security issues for a site containing a highly radioactive source.

However isotope based sources do have advantages for fixed site facilities. If such a fixed location treatment irradiation facility was also of value this alternative should be considered by the mortuary community. An example would be a sterilization facility located at Dover AFB. Electrical power consumption is lower than for electron sources as the power requirements are primarily for material handling. Large field sizes with very high dose rates with very high penetration are possible. The advantages for such types of facilities for in fixed site facilities are indicated by their extensive use for industrial sterilization.

A limited listing of manufactures of equipment and their websites is in the appendix to illustrate that a number of manufactures exist that potentially could provide the type of sources necessary.

Regulatory Issues

By analogy to industrial processing there are several sets of approvals, regulator environments, which must be addressed for the irradiation facility to come on line.

First is the approval that radiation is appropriate and will provide the degree of sterilization and safety post treatment required. Second, is that the radiation exposure does not cause unacceptable changes that affect the safety, for handling or use, of the material post-processing. Third, the design of the facility must adequately protect the safety of the operators and non-operational personnel. Lastly, the defining periodic process of inspection, testing and verification that the facility continues to be operated as approved, both for the effective sterilization of materials and its safety.

Determining which civilian or DoD agencies have the responsibility and authority for oversight certification and approval will be an important aspect of brining this technology to the field.

The following are agencies and groups who may fill each of these roles. As for medical device and foods there is a well established regulatory process it is reasonable to suppose that analogous procedures will be necessary for approval and on going use of any method for remains decontamination.

Certification of private sector industrial sterilization processes is done by the Food and Drug Administration Division of Radiological Devices. They work closely with the American Association of Manufactures of Industrial Devices to set standards for safety assurance levels, the total radiation dose for adequate treatment, the specific testing methods and criterion for both initial approval and quality control and assurance. They approve the safety of devices and machines that produce radiation for use with humans, including isotope based irradiators and X-ray machines, such as those used for medical diagnoses and treatment. They also set and approve standards for operator training and certification.

The National Bureau of Standards and Technology (NIST) set standards for measuring radiation doses (radiation dosimetry).

The Nuclear Regulator Commission (NRC) monitors and approves the use of many types of radiation sources and the monitoring of personnel for radiation exposures. The NRC approves the design, safety and operation of isotope based radiation sources, including their installation and fueling.

Summary of State of the Art of Using Ionizing Radiation to Treat Contaminated Human Remains

The following statements can be made about the state of the art for the use of ionizing radiation for sterilization of human remains:

1. Data on the radiation sensitivity of many microbial pathogens, including putative biological threat agents, are available.
2. The technological base is well developed for commercial irradiation of a wide variety of materials to effect sterilization.
3. A previous survey concluded that a high energy electron source could fulfill the requirements of a transportable system capable of biological decontamination of materials including mortuary remains. There are commercially available high energy electron-producing machines that are small and robust enough to be transported. These machines offer the important advantage that when not turned on, no radioactivity is available to pose a hazard or require security for radioactive materials.
4. The approach has been validated for the inactivation of spores in a human phantom covered in Kevlar body armor, placed in a body bag inside a 16-gauge steel container (simulating a Ziegler case).
5. The Armed Forces Epidemiologic Board determined that this approach met the military and CDC requirements for return of remains contaminated by *Bacillus anthracis* (Appendix 1).

Recommendations

- As early as possible identify the best case and the next best acceptable specifications from the mortuary community.

This includes the environments for operation (wet, cold, hot), the degree of portability or transportable, including explicit information on the weight and size. The maximum through put e.g. numbers of remains and time for treatment of each. What the condition

of the remains are likely and possible. What other materials, clothing, armor, containment will also be exposed.

- Identify the regulatory groups and agencies which have approval authority.

This includes certification of the efficacy of the process, safety of the radiation source and site design, out year testing, approval and certification of safe and effective operation, training and certification of operators, monitoring the safety of operators and personnel during use.

- Determine the safety assurance level necessary (SAL).
- Determine whether currently known microbial radiation sensitivity values (D_{10} values) are adequate for design and operational planning. Alternatively decide whether additional remains specific testing to provide more accurate D_{10} values is necessary or desirable.
- Determined based on the SAL and current estimates of D_{10} values the appropriate total dose.

Previously 120 kGy was used for scoping calculations, but more recent research suggests this may be higher than necessary.

- Determine the current state of radiation devices characteristics, with emphasis on commercial off the shelf high energy electron sources with X-ray conversion capabilities. Specifications to be evaluated include but are not limited to size, weight, beam direction, power requirements.

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APPENDIX

Organizations and Enterprises

This is not intended to be exhaustive nor an endorsement of any particular group or equipment manufacturer. An internet search of potential vendors provides many more potential suppliers of this type of equipment. These are provided as illustration that radiation sterilization is a well established industry with multiple suppliers of equipment and services and with well established regulatory processes and responsible agencies.

Regulatory

Association for the Advancement of Medical Instrumentation

<http://www.aami.org/>

Trade group which provides extensive good practice guide lines and information processing and sterilization including radiation based methods.

Food and Drug Administration, Center for Devices and Radiological Health

<http://www.fda.gov/Radiation-EmittingProducts/default.htm>

They regulate all radiation producing products used and produced in the US.

International Atomic Energy Agency

<http://www.iaea.org/>

National Institute of Standards and Technology

<http://www.nist.gov/index.html>

Set and are reference standards for radiation measurements. Conduct research in radiation dosimetry. Radiation source services provided for research and development.

Equipment Suppliers and Consulting Services

Representative examples and links containing links to other suppliers

IBA

<http://www.iba-worldwide.com/>

Radiation Safety Academy

<http://www.radiationsafetyacademy.com/radsvcs.html>

L3 Communications Applied Technology and Pulse Sciences

<http://pulsesciences.com/TitanScan/index.html>



DEPARTMENT OF DEFENSE
ARMED FORCES EPIDEMIOLOGICAL BOARD
5109 LEESBURG PIKE
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AFEB

JUN 10 2003

MEMORANDUM FOR Assistant Secretary of Defense (Health Affairs)

SUBJECT: Disposition of Irradiated Remains - 2003-10

1. References:

- a. Memorandum, Deputy Assistant Secretary of Defense, Clinical and Program Policy, 8 April 2003, Disposition of Irradiated Remains.
- b. Memorandum, Armed Forces Epidemiological Board, 14 January 2003, Disposition of Contaminated Human Remains – 2003-06.
- c. Joint Publication 4-06, "Joint Tactics, Techniques, and Procedures for Mortuary Affairs in Joint Operations.
- d. Joint Procedures for Decontamination and Disposition of Human Remains in Operation Desert Storm, 25 April 1991.

2. A workgroup of the Armed Forces Epidemiological Board (AFEB) met by teleconference on 29 April 2003 to consider a request submitted to the Board by the Deputy Assistant Secretary of Defense for Clinical and Program Policy to review a proposed irradiation procedure for decontamination of anthrax contaminated human remains. The Board had previously made recommendations on the safe handling and disposition of human remains potentially contaminated with biological warfare agents and the requirement for return to the United States in a hermetically sealed container to comply with current Code of Federal Regulations requirements. Subsequent evaluation and testing by DoD of available hermetically sealed systems for transportation of potentially contaminated human remains from an operational theater were unacceptable because of the inability to maintain the hermetic seal at altitude and assure maintenance of the seal in an operational environment.

3. To address the question before the Board, the workgroup met with scientists from the Armed Forces Radiobiology Institute and the National Institute of Standards to review current irradiation technology and research addressing application of high-energy x-ray radiation to sanitization of bodies contaminated with biological warfare agents. Kill curve data for anthrax spore surrogates in human phantoms wrapped in body armor and contained within 16-gauge stainless steel containers were presented. Based upon the data presented, discussions with subject matter experts, and

AFEB

SUBJECT: Disposition of Irradiated Remains - 2003-10

consideration of unique military operational concerns, the following recommendations are made concerning the question to the Board related to the proposed irradiation procedure for decontamination of anthrax contaminated human remains:

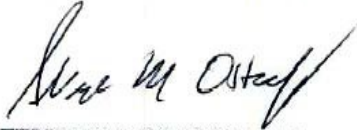
- a. The proposed process of using of high-energy x-ray radiation to destroy both vegetative cells and spores of *Bacillus anthracis* in contaminated human remains appears effective as tested and evaluated by the staff from the Armed Forces Radiobiology Institute and the National Institute of Standards.
- b. The use of dosimeters to verify proper dose delivery should be a standard practice to verify that a radiation dose that will kill both vegetative cells and spores of *Bacillus anthracis* has been delivered.
- c. Requirements for importation into the United States of human remains potentially contaminated from anthrax, including return in a hermetically sealed container and issuance of a import permit for the remains, once treated, would no longer be required as the remains would no longer be contaminated and thus not present a hazard from anthrax.
- d. Procedures for the safe handling and temporary storage of potentially contaminated human remains, as recommended in Armed Forces Epidemiological Board memorandum of 14 January 2003, Disposition of Contaminated Human Remains -2003-06, should be followed.
- e. As the high-energy x-ray decontamination system will pose a potential occupational health hazard, appropriate safety procedures and personnel monitoring should be employed.
- f. No information was presented concerning agents other than *Bacillus anthracis*. There are data to suggest that viruses may not be as susceptible to irradiation technology as more complex microorganisms such as bacteria. Therefore, It is important to conduct additional research to document efficacy against other possible biowarfare agents, particularly viruses such as smallpox, with appropriate surrogates when possible.

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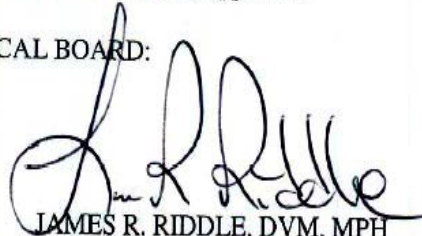
SUBJECT: Disposition of Irradiated Remains - 2003-10

4. The above recommendations and observations were unanimously approved.

FOR THE ARMED FORCES EPIDEMIOLOGICAL BOARD:



STEPHEN M. OSTROFF, MD
AFEB President



JAMES R. RIDDLE, DVM, MPH
Colonel, USAF, BSC
AFEB Executive Secretary

Enclosure

Memorandum, Deputy Assistant Secretary of Defense, Clinical and Program Policy, 8 April 2003,
Disposition of Irradiated Remains.

CF:

Board Members and Consultants (w/encl)

J4-MRD (w/encl)

ASA (M&RA) (w/encl)

OASD(HA)/C&PP (w/encl)

AFRRI (w/encl)

Library of Congress (w/encl)

SAAA-PPO (w/encl)

Microbial Decontamination Background Information

Introduction

Successful elimination of microbes depends on several factors which broadly are a) the characteristics of the microbes, b) the characteristics of the antimicrobial agent or process, and c) the efficacy of interaction. Additional factors which influence the methods used include the level of decontamination, sterilization versus sanitization, and the preservation or damage to the contaminated material that is acceptable. Measurement that the effective level of antimicrobial has reached all areas to be cleaned is also necessary.

Methods for sanitization and/or sterilization to remove microbes have been an important aspect of applied microbiology from their first discovery and understanding that they are the causative agents of food spoilage and disease. The field is well established, and there is a robust literature, well defined national and international regulations and methods of standard practice. Extensive studies have established best practice methods for removal or killing of bacteria, bacterial spores, viruses and fungus for a broad spectrum of physical environments, materials and products. Particular emphasis is in the areas of food preservation, medical device manufacture and re-use, and medical care and research laboratory environments. Citation to both important general references and standards are listed in the literature cited.

The terms sanitization and sterilization have specific meanings in this context. Sterilization is to eliminate all of all viable growth by microorganisms. Reappearance of viable growth does not occur at any time after treatment as long a no new microbes are introduced. Other terms such as sanitization refer to a reduction in the number of organisms, but not complete elimination of all viable organisms. Cleansing likewise is removal but the level of residual viable microorganisms is unspecified. Decontamination is also less specific term, often used in reference to removal of microbes from an environment where they have been actively introduced. For a specific context it is important to evaluate whether the goal of a particular decontamination process is sanitization or sterilization.

Differing types of microorganism, different species of the same microorganism, or their biological state, free living or spore form all can have different sensitivity to any and all methods of killing. For example for most bacteria, heat, chemicals, and radiation, vegetative, free living bacteria are much more vulnerable than spores. The effectiveness of antimicrobials can dependent on additional physical or chemical factors. For example moist heat is generally effective at lower temperatures than dry heat and chemical agents often are more effect at a specific pH or in hydrated versus anhydrous environments.

The ability to have high assurance that the antimicrobial agent can reach all portions of the area or object to be sanitized or sterilized is an obvious requirement, but in practice can be an important problem. Complex geometries or closed volumes or even the rougosity of open surfaces can all provide protection to microorganisms from antimicrobial agents. Process control requires that the antimicrobial level can be measured to assure that treatment is adequate.

Brief descriptions of approaches to illustrate the above points follow. Medical waste is routinely treated with high temperature dry heat in specially designed incinerators. This is extremely

effective for complete elimination of pathogens, as typically only water, carbon dioxide and metals are the output, but of course results in complete destruction of the materials. Autoclaving, high pressure moist heat, is commonly used in laboratories and hospitals to sterilize glass and metal as they are not damaged and it is rapid and highly effective as long as care is taken that there all surfaces are accessible. In practice special permeable bags and open or partially open vessels are used. There is high assurance that treatment was adequate as temperature and pressure are easily measured. But the materials sterilized are such things as liquids, glass, stainless steel and fibers that can withstand wetting and elevated temperatures. Gaseous or vapor disinfectants chemicals can be dispersed in complex pieces spaces or objects and for materials like hydrogen peroxide do little or no damage. However, for most to be fully effective specific temperature or humidity conditions are necessary. Neither of these methods is effective for closed containers. However, even for very densely packed materials vapor or gaseous disinfectants can be limited by inconsistent and incomplete penetration and therefore ineffective. Chemical concentrations measurements are generally not as fast or readily done as physical measurements and accurate sampling in the interior of complex geometries difficult. Liquid agents are easily used and can be quite effective and routinely used on accessible environmental surfaces. But commonly used ones such as neutral chloride solutions must be made shortly before use to be at full, known strength and can be damaging to many materials.

Ionizing Radiation Industrial Use

Ionizing radiation has several advantages. First is its high penetration through most materials and second it is readily and quickly measured with high accuracy allowing verification that the desired exposure dosage was delivered to the target material. Also it is generally considered a low temperature process, in contrast to heat, moist heat and some chemically based methods.

Radiation sterilization has been used in both the food and medical industries for approximately 40 years. Almost all sterile plastic disposable items used in clinics and research laboratories are radiation sterilized. Due to the high assurance of penetration large quantities of bulk material with or without complex geometry can be processed. The process of food irradiation has been approved for human use in over 40 countries and more than 60 food products. A survey of the food technology literature results in hundreds of scientific papers to establish the effective ionizing radiation dose for specific foods under a wide variety of specific physical and chemical conditions. Examples of three of the common current applications are irradiation of spices to eliminate both insect and bacterial contamination, irradiation of ground meats, particularly beef in part due to the problems associated with highly pathogenic E.coli and milk, "cold pasteurization" to allow ambient shelf storage. Irradiation is also used in the agricultural industry to extend shelf life of fruits and vegetables or prevent sprouting of root crops.

Other applications, medical and non-medical are illustrative of the maturity of the technology. Polymerization of plastics is an extensive application for the production of films, e.g. plastic wraps and plastic polymer coatings or linings. Some facilities can be very large such as those which process industrial pipe cladding or linings being able to handle long large diameter pipe sections. Radiation oncology applications illustrate the precision which doses can be delivered to a specific volume, of tissue, with the goal of salvaging surrounding tissues.

Currently the IAEA lists approximately 3000 cobalt and cesium based irradiators worldwide. Standards for the construction of such facilities and their safe operation are well established and under the specific country's regulation and IAEA supervision. This is a low estimate of the numbers and types of irradiation facilities as other types of radiation sources used for these applications, e.g. linear accelerators, which do not use radioisotopes, and therefore not included in this listing.

Total Radiation Dose for Decontamination

The total radiation dose to be used is determined by the level of contamination (bio-burden), the safety assurance level desired and the radiation sensitivity of the microorganisms present. Each of these is discussed in turn.

Bio-burden

For the sterilization of human remains the levels of contamination potentially will be high, reaching the maximum that bacteria, bacterial spores and viruses can obtain in culture or even higher especially externally, if significant concentration of materials is done prior to their release. The specific concentrations are dependent on the details of microbe growth characteristics and the specific conditions used and processing and dispersion conditions. The following values are provided to provide general information on the magnitudes of organism, and therefore the levels of decontamination necessary. Typical culture growth level that can be readily obtained for bacterial and bacterial spores is 10^8 colony forming units per gram (or milliliter). Virus preparations can be as high as 10^9 plaque forming units per gram (or milliliter). Concentration and/or drying can increase these concentrations easily by 10-10,000 fold. While post processing dispersion may decrease absolute concentrations, and therefore external contamination could be lower, the infectious disease process will increase number back towards or two their equilibrium growth levels. Therefore any sanitization or sterilization process to be effective must reduce bio-burdens of viable microorganism per unit weight or volume on the associated clothing and materials and the remains by values on the order of at least 8-12 logs. When safety assurance levels are included, as discussed below, the log reduction necessary is even greater.

Safety Assurance Level

The target level for residual levels of microbial contamination is ultimately a statistical statement of risk. It is not unique to radiation sterilization, but a necessary standard for any method.

The "SAL" is best based on medical considerations taking into consideration both the virulence of the microbes of concern and the number (estimated infectious dose; EID) necessary to cause disease.

Typically the amount of treatment specified is to reduce the likely concentration of infectious agent several orders of magnitude below either the EID level or to or below the zero organism per gram (or milliliter) level. For example a treatment that reduces the concentration three logs below zero per gram has reduced the concentration on a probability basis to 1 organism per 1000 grams.

The following example illustrates these points. Consider material that has been contaminated with 10^{10} per gram of microorganisms. The particular agent has an EID₅₀ of 100, that is that 100 viable

microbes will cause disease in 50% of people exposed. If other medical or regulatory guidance is that 1 viable organism per 10 kilograms would be a safe level this is equivalent to saying that the safety standard required is that an individual would need to be exposed to 10,000 grams of material in order to receive one infectious dose. For the initial concentration in this example to reach this level would require 10 logs plus 4 logs or 14 total logs of reduction in microbe concentrations.

In industrial food and medical device processing a typical “rule of thumb” has been a 12 log reduction in bio-burdens, with the assumption of 10^6 cfu or pfu per gram of initial contamination e.g. a 6 log reduction beyond the no viable organisms per unit concentration. However this is likely not to be the best a starting point for the mortuary application. The bio-burdens are likely to be higher, as discussed above, the virulence of the microorganisms maybe higher then those in commercial industrial settings, and include those biological agents for which there are no vaccines or other medical treatments.

Radiation Sensitivity of Biological Agents

Comparisons of the sensitivity of microbes to ionizing radiation under different biological, physical and radiological conditions are most readily and routinely reported in the scientific literature using the D_{10} or k value. The D_{10} is the dose of radiation necessary for a one log reduction in viability. Typically this value for the conditions of interest is determined from measuring the loss in viability with increasing doses of radiation exposure. Formally the resulting curve is the decimal dose reduction curve, k is the slope of the curve and D_{10} is the reciprocal of the slope. The unit for radiation dose is the Gray (Gy) but as microorganisms are radiation resistant units are in kilo Gray of dose (kGy).

D_{10} values in general as follows:

- Less than 20 kGy for all types of microorganisms
- Vegetative bacteria are less than 1 kGy; with exceptions up to 10 kGy
- Bacterial spore D_{10} ranges from 1-10 kGy
- Viruses range from 1-15 kGy.

The above values are based on extensive review of literature on the radiation inactivation of microbes based on references in both the open academic and DoD databases. Most information is for bacteria and bacterial spores, with less on viruses and a very small number of reports for fungi. The radiation sensitivity values are generally established for contamination of various types of foods, suspensions of microbes in water or saline, and on hard plastic or metal surfaces. Most information for the conditions of exposure and the specific organisms used reflects largest area of interest, industrial and food technology applications.