Boston Public Health Commission

Biological Laboratory Safety Permit Application

SECTION 6: BSL-4 LABORATORY DECONTAMINATION PLAN

Boston University National Emerging Infectious Diseases Laboratories

September 2014

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1.0 PURPOSE AND APPLICABILITY

The purpose of the Laboratory Decontamination Plan for the National Emerging Infectious Diseases Laboratories (NEIDL) located at Boston University is to clearly define established procedures that represent a critical safety barrier in Biosafety Level 4 (BSL-4) laboratories. The plan also identifies the individuals responsible for upholding these procedures and for providing oversight, as well as the protocol(s) to be followed to ensure the efficacy of decontamination efforts.

2.0 ROLES AND RESPONSIBILITIES

2.1 Director, Research Safety, Environmental Health & Safety

The Director, Research Safety of BU's Environmental Health & Safety department (EHS) is responsible for the management of the NEIDL Research Safety Program. The Director provides oversight of all decontamination functions including: 1) the determination of appropriate decontamination methods for laboratory and emergency response applications, and 2) training and competency evaluations of NEIDL staff who have responsibility for the execution of decontamination protocols.

2.2 Principal Investigator

The Principal Investigator (PI) is an authorized individual approved by the CDC Select Agent Responsible Official (RO) who is responsible for the scientific and technical direction of a select agent or toxin project or program. Decontamination responsibilities of the PI include ensuring that all authorized individuals under his or her direction comply with and maintain all BUMC decontamination provisions such as: 1) successful completion of decontamination safety training and drill exercises; 2) reporting all incidents, accidents, and exposures related to select agent or toxin materials to the CDC Select Agent Responsible Official and the Research Occupational

Health Program; 3) proper decontamination of materials, rooms, etc., following all normal operations as well as following any incident; and 4) proper decontamination of BSL-4 suits.

2.3 Responsible Official

The Responsible Official is an authorized individual with responsibility, authority, and control to ensure compliance with the Department of Health and Human Services (DHHS) and U.S. Department of Agriculture (USDA) Rules and Regulations pertaining to the possession, use, and transfer of select agents and toxins. BU designated the Director of Research Safety to serve as the CDC Select Agent RO. The CDC Select Agent RO will ensure the proper decontamination of all select agent materials. In the event of an incident, the CDC Select Agent RO will immediately inform the Associate Vice President for Research Compliance (AVP-RC), oversee the proper decontamination of materials or rooms involved, and report to the DHHS and the USDA the results of these efforts.

2.4 Associate Vice President for Research Compliance

The Associate Vice President for Research Compliance (AVP-RC) is responsible for the oversight of the control of hazards in the research laboratories and for ensuring that comprehensive, enterprise-wide programs are in place for the safe handling of all hazardous materials (e.g., biological, chemical, radiological). The AVP-RC serves as the Responsible Official for all compliance functions associated with the City of Boston's Public Health Commission Biological Laboratory Regulation.

2.5 Director, NEIDL Facilities, Facilities Management & Planning

The Director of NEIDL Facilities is responsible for coordination of the decontamination of all facilities and equipment and BSL-4 suit decontamination. This coordination will involve facilitating an annual recertification of critical systems that support the BSL-4 laboratory (i.e., HVAC/HEPA systems, breathing air, effluent decontamination system and backup generator) and emergency response applications. The Facilities staff will monitor the status of all support systems including gas, steam, vapor, filtration, chemical, and heat.

2.6 BSL-4 Suite Authorized Individuals

All authorized individuals in BSL-4 suites will abide by all requirements set forth by the PI, RO, AVP-RC, and EHS. This will include compliance with all safety training, routine decontamination research practices, including BSL-4 suits and emergency response decontamination applications.

3.0 DECONTAMINATION METHODS

3.1 Basic Concepts

Decontamination is a critical step in any BSL-4 laboratory operation to ensure that hazardous microorganisms have been destroyed when no longer pertinent to a study. Decontamination is disinfection or sterilization of infected articles to make them suitable for use. Disinfection is the reduction of undesirable microorganisms in order to prevent their transmission. Sterilization is the elimination of microorganisms from a set domain (i.e., space) or given entity (i.e., supplies and equipment).

Inactivation of microorganisms can be achieved through four different means:

1. Heat Treatment – exposure to elevated temperatures for a set time.

2. Chemical Treatment – physical contact between a liquid, gaseous, or vaporous chemical and the surface of a material.

3. Irradiation – refers to any radiation process in which the individual quanta of radiated energy are able to ionize atoms or molecules of the substance in which the energy is absorbed. This leads to chemical changes that can damage biological tissues and structural materials.

4. Incineration – the complete combustion and oxidation of solid-state organic matter.

Selection of the appropriate method for the inactivation of biohazardous agents primarily relies on the nature of the organism and the type of material involved in the inactivation. Susceptibility of the agent to the inactivation method and efficacy will be determined prior to use of the agent. Other factors are also determined including the compatibility of the inactivation method with the personal protective equipment used in the space, its effects on the biocontainment systems and containment surfaces and laboratory equipment and others. EHS works with the laboratory to assess the agents, materials and procedures involved to determine the most effective method. Depending on variable factors such as agents, materials, equipment, areas, etc., to be decontaminated, principal methods of decontamination will be developed to achieve disinfection and/or sterilization. The methods are based on the appropriate chemicals, methods, and length of treatment times that have proven efficacy against the agents being treated.

3.2 Principal Methods

3.2.1 Autoclaving

Infectious laboratory solid and semi-solid wastes, along with various laboratory supplies and equipment can be effectively sterilized in a "pass-through" type autoclave chamber utilizing steam heat treatment to destroy microorganisms. There are two types of autoclaves available in NEIDL: Small laboratory autoclaves (Steris SV-120) and large rack autoclaves (Steris Finn-Aqua). Each is capable of running pre-vacuum cycles. The autoclaves have an extremely sturdy but flexible bioseal that is secured to the inner wall to ensure the containment barrier is maintained, and each autoclave is fitted with interlocked doors. The interlock prevents both doors being open at the same time and thereby maintains the containment barrier. The chamber of the autoclaves is equipped with HEPA filtration systems to filter air discharged from the chamber during the pre-vacuum cycle. Autoclaves work by allowing steam heat transfer, or saturated steam penetration to a waste load for a given cycle time. Operating parameters (e.g, 122°C for 60 minutes, or 126°C for 120 minutes) for autoclaves are set by establishing standard load sizes and types and validating the cycles, using thermocouples and/or biological indicators placed within test loads.

3.2.2 Chemical Disinfection

Chemical Disinfection of solids is the act of physically allowing a liquid chemical to contact the surface of an impermeable solid object (e.g., stainless steel table top). A liquid chemical is typically applied in a manner similar to that of a household-cleaning product, such as a spray bottle and brush, or a mop and bucket. After application of a liquid chemical on a surface for the contact time (determined through manufacturer's specifications and verified in-house with the specific BSL-4 agents) the treated surface is typically rinsed of residue with water that is then conveyed through a sink or floor drain to the liquid effluent decontamination system for redundant treatment. An example of this method of chemical sterilization is the Chemical Disinfectant Shower System in which the researchers wearing positive pressure suits pass through the chemical shower sequence to effectively decontaminate the outside of their suits before they exit the BSL-4 space.

Chemical Disinfection of liquids is the act of physically mixing a liquid chemical with a potentially contaminated liquid waste solution. The specific ratio of solutions, extent of mixing, and contact time are predetermined through validation with biological indicators. This process can be performed in a container prior to discharging to the liquid effluent decontamination system.

3.2.3 Gaseous and Vapor Decontamination of Spaces

The gaseous treatment of sealed BSL-4 rooms is performed when it is necessary to ensure thorough decontamination of all areas within the space, including difficult to access spaces behind equipment or within component panels. The type of scenarios warranting gaseous decontamination can be: annual recertification of the space, scheduled maintenance work, the decommissioning of a working laboratory, or decontamination of a spill that cannot be effectively cleaned using a surface chemical treatment. One process of gaseous decontamination consists of the depolymerization of paraformaldehyde (a carcinogenic substance) by heat, such as an electrically heated plug-in device, and the presence of water to maintain the desired relative humidity level. Formaldehyde vapor effectively diffuses throughout the space and makes contact with every exposed surface. Validation of the decontamination process is achieved with biological indicators. An important preceding action to this gaseous decontamination is preparation of the room by opening equipment panels, removing lighting lenses, and removing all absorbent materials (such as cardboard) to expose all surfaces. Established parameters of this process are an ambient temperature of at least 21°C, relative humidity of at least 70%, a paraformaldehyde application rate of no less than 10.6 g/m^3 (0.3 $grams/ft^3$) and a contact time of not less than eight (8) hours. Prior to ventilation of the space after the decontamination cycle contact time, the formaldehyde is neutralized with ammonium carbonate or ammonium bicarbonate at 1.1, or 1.5 times (respectively) the mass of paraformaldehyde initially applied.

Vaporized hydrogen peroxide (VHP) can also be used for decontamination of spaces. VHP is safer to use, is non-carcinogenic, and environmentally friendly in contrast to paraformaldehyde. In the cold sterilization process, 35% liquid hydrogen peroxide (300,000 parts per million [ppm]) is vaporized to yield concentrations of at least 250 ppm to over 1,000 ppm. The process is effective at temperatures ranging from 4° to 80°C, and the area to be decontaminated should be conditioned, ideally, to have a relative humidity not greater than 30%.

Chlorine Dioxide gas is also an appropriate method for decontamination. The gas has penetrative properties quite similar to formaldehyde and can be easily dispersed throughout the laboratory space or equipment compartment, such as the Biological Safety Cabinet (BSC). It can penetrate through the High Efficiency Particulate Air (HEPA) filters of the BSC, making it an efficient way to decontaminate the HEPA filter surface and substrate. Use of Chlorine Dioxide gas also presents several advantages. It has sporicidal properties similar to formaldehyde gas; decontamination time is shorter compared to formaldehyde gas; it does not leave any residue; and it is not carcinogenic. However, the gas has corrosive properties

and so its use for certain surface applications needs to be evaluated properly to ensure materials compatibility.

3.2.4 High Efficiency Particulate Air (HEPA) Filtration

The BSL-4 exhaust air ducts are equipped with two HEPA filters in series. HEPA filters have an efficiency of greater than 99.97% for retention of particles of a nominal size of 0.3 microns (this is the most penetrating particle size; the efficiency of particle retention is greater than 99.97% both below and above this size). The particle capture mechanisms involve inertial impaction, interception, diffusion and electrostatic attraction. The exhaust air from the BSL-4 rooms passes through two HEPA filters in series before being discharged to the environment. The filters are located in pressure-tested sealed housings in the interstitial mechanical support space. The housings are designed to allow for filter scanning to determine filter integrity, and are fitted with magnahelic gauges to display the pressure differential across the filter and thereby assess filter loading. Monitoring of the pressure gauges will alert the operations and maintenance staff to when it is necessary to replace the HEPA filters. Airtight dampers isolate the filter housing in preparation for decontaminating the HEPA filters prior to filter removal and replacement. The decontamination procedure involves introducing gaseous decontaminants such as formaldehyde gas into the isolated housing through injection ports. The formaldehyde gas decontaminant is maintained for a predetermined cycle, as determined during initial validation. On completion, the housing is safe to open, and removal and replacement of the filter can proceed.

3.2.5 Liquid Effluent Treatment System

The BSL-4 rooms are equipped with stainless steel sinks and floor drains to receive chemically decontaminated liquids generated through routine laboratory activities (e.g., floor washing with Microchem disinfectant and subsequent rinsing). These drains will also be used to dispose of decontaminated liquid substances generated in the biological safety cabinets (e.g., virus stocks). No known or perceived contaminated substances will be disposed of through these drains without first being decontaminated by laboratory personnel. As a secondary measure of safety, the sinks and floor drains are plumbed to the effluent decontamination system. Downstream of the BSL-4 sinks and floor drains are three 1,500 gallon liquid effluent treatment tanks. Through a pressurized steam-heat transfer jacket, the temperature of the waste in the inner chamber effluent treatment tank is elevated to a validated sterilizing temperature for a predetermined cycle (e.g., 121°C for 60 minutes; the cycle is programmed for 123°C to achieve 121°C for 60 minutes based upon the initial EDS

cycle validation). After completion of the treatment cycle, the liquid is cooled and discharged to pH neutralization system.

3.2.6 Irradiation

Irradiation is used primarily to inactivate pathogens for study at lower containment levels. It may also be used to decontaminate small solids, semi-solids, or liquid substances. It is the preferred method of decontamination for materials that are sensitive to heat and/or chemical treatment that will be used for further research. Items associated with laboratory research that warrant this type of decontamination include solvents, plastic instruments, or biological material such as tissue cultures whose organic make-up would be damaged through another means of sterilization. The irradiation process begins by placing materials in a shielded chamber and then exposing them to a timed dose of gamma irradiation. Gamma irradiation, produced by a radioisotope such as Cobalt-60, penetrates the mass of the materials transferring small amounts of high intensity energy to the material's molecules through the movement of electrons. This process destroys the viability of any microorganisms. Successful inactivation of microorganisms by gamma irradiation is determined by generation of a "kill curve" or dose-response curve. That is, exposing the agent to a defined dose of gamma irradiation and then culturing the agent to determine viability.

3.2.7 Final Disposal of Solid Wastes

Following decontamination by autoclaving (and successful verification of the efficacy of decontamination), solid and semi-solid wastes are double-bagged and placed into a leak-proof transport container. All autoclaved animal carcass wastes are disposed of on-site by tissue digestion (alkaline hydrolysis). Autoclaved animal bedding is packaged as medical waste. Medical waste shipping containers will meet or exceed the U.S. Department of Transportation medical waste shipping and packaging requirements (United Nations #3291). Once properly packaged, the shipping boxes will be catalogued into appropriate shipping manifests and transported by an authorized third-party biowaste handler for final disposal.

3.3 Validation and Verification

It is important to specify the difference between validation and verification processes.

Validation is a rigorous process by which the various decontamination methods and cycles are established as producing at least a 6 log reduction of resistant microbiological materials (e.g., bacterial test spores), or meeting other agreed upon criteria (e.g., see animal carcass autoclave validation). Typically there are two steps: a temperature mapping (e.g., to ensure uniform heating

of steam heat equipment including autoclaves and EDS tanks), and then microbiological challenge using indicators such as *Geobacillus stearothermophilus* spores (at a population exceeding 10^6). The requirement is for 100% kill on each of three consecutive decontamination runs. Typically validation processes only need to be repeated if there are major repairs to equipment or significant changes within the building (e.g., large changes in supplied steam pressure).

Verification is the continued confirmation on a scheduled basis that the decontamination process or cycle remains effective as originally validated. Indeed, successful verification confirms that a specific decontamination process has destroyed microorganisms within the waste biological material(s). All *principal methods* of decontamination undergo routine and documented verification processes e.g., the EDS tanks are each verified on at least a quarterly basis, while laboratory autoclaves are verified on every run. The verification process for a particular method of decontamination varies (refer to 3.1 “Basic Concepts”).

3.3.1 Validation

To validate any decontamination process, cycle parameters must be established in advance. These parameters take into consideration the material being decontaminated, their size and state, as well as exposure time, and other physical requirements (i.e., temperature, chemical concentration, etc.). Typically, a biological indicator is then used to validate the cycle using a specific set of physical parameters.

3.3.1.1 Autoclave

To validate autoclave cycles, the autoclave is first temperature mapped using calibrated dataloggers. This is to determine if any specific regions of the chamber are cooler than others (and therefore likely to be less efficient at decontaminating loads). Any “cold spots” must be taken into consideration with respect to load placement. Next, representative maximum load configurations are prepared. In the case of laboratory solid wastes, reusable laundry items (scrubs, towels etc.) and cages, bedding and bottles, dataloggers are placed into representative loads (to check the temperature achieved in the load) as well as two biological indicators per load (*Geobacillus stearothermophilus* spores at a population exceeding 10^6). The autoclave must inactivate the biological indicators on each of three consecutive validation runs. In the case of animal carcasses, thermocouples are placed into representative carcasses in specified batches (e.g., 5 mice, 10 mice, or 20 mice) and state (e.g., refrigerated, frozen etc.). Three consecutive runs are performed and the longest time taken for any carcass thermocouple to reach 121.1°C is recorded. To this is added 60 minutes (“sterilize”

time) plus a safety margin (this is sufficient to ensure an "overkill" cycle is established and utilized). The rationale is that one hour at 121.1°C reliably decontaminates items, so if the time for the coldest region to reach 121°C is used as "time zero" the cycle can be expected to be effective if it is greater than 60 minutes beyond time zero. This would then be confirmed using biological indicators. For animals larger than mice, self-contained biological indicators may be impractical as the vents would tend to clog with melted fat. In this case, a significant safety margin (i.e., additional time) would be put into effect to ensure complete and effective "overkill".

3.3.1.2 Space

For space decontamination validation (using VHP or formaldehyde) the room is prepared (receptacles opened, organic materials removed and water sources capped or drained). The room is then conditioned (for VHP this means reducing the humidity to 30% or less and for formaldehyde this would mean increasing the humidity to at least 60%). Biological indicators, at least 1 per 100 ft^2 (typically 30 in a standard BSL-4 lab), would be deployed around the room (at various heights). Chemical indicators may also be deployed to obtain immediate visual proof of penetration of the gas/vapor to all parts of the space. In the case of VHP the concentration of the vapor is monitored. The level that NEIDL has established as required is >250 ppm for 3 hours. In the case of formaldehyde, this is standardized as 0.3g of paraformaldehyde per cubic foot, and it is assumed that all of this converts to formaldehyde when heated (provided there is nothing left in the pan after "the burn"). The biological indicators are collected after decontamination and aeration, and processed. After collection of the biological indicators, (for validation or any subsequent space decontamination) absolutely **no entry is permitted** for any personnel until the success of the decontamination has been confirmed. The validation criterion is zero growth of any of the biological indicators and this must be achieved on each of three consecutive decontamination cycles.

3.3.1.3 Filters

HVAC HEPA filters and housings are decontaminated using the Nextek formaldehyde gas generator fitted with a supplementary blower to circulate the gas injecting on the "dirty" side of the housing and extracting on the "clean side" of the filter. Biological indicators are placed throughout the housing upstream and downstream of the HEPA filters. These biological indicators are retrieved and processed. Zero growth of the biological indicators is permitted, and this must occur on each of three consecutive decontamination runs.

Plumbing vent filters and housings and all of the small HEPA filters are

decontaminated using chlorine dioxide in a closed loop using a small circulator, injecting on the "dirty side" of the housing and extracting from the "clean" side). Biological indicators are inserted into the return hose. As above, these biological indicators are retrieved and processed. Zero growth of the biological indicators is permitted, and this must occur on each of three consecutive decontamination

3.3.1.4 Effluent Decontamination System

Each of the three EDS tanks are temperature mapped using thermocouples attached to a frame that is inserted into the body of the tank. Alongside each thermocouple is a canister into which a biological indicator is placed. The validation criteria are that during the sterilization phase each thermocouple must register at least 121.1°C, maintain this minimum temperature for one hour, and do this on three consecutive runs.

3.3.1.5 Gamma irradiation (inactivation)

The validation of an irradiation cycle to inactivate lipid enveloped viruses is initially performed using a surrogate organism for hemorrhagic fever viruses. Specifically, Vesicular Stomatis Virus (VSV). Known concentrations of different viral suspensions are placed into sealed containers and irradiated at different dosages to develop a kill curve. The test samples include non-irradiated control samples (which remain viable). The test samples and control samples are analyzed and tested to determine that the surrogate agents have been completely inactivated. As different hemorrhagic fever viruses are added for research, the irradiation cycle will be confirmed using the actual virus.

3.3.1.6 Tissue digester

It is important to note that the tissue digester is used for carcass disposal only. All carcasses are autoclaved using a validated cycle. However, validation of microbiological inactivation can be performed on the tissue digester by inserting biological indicators (e.g., MagnaAmp) into canisters and attaching them to various locations on the digester basket. After a digester run, the canisters and biological indicators can be retrieved and processed. A successful validation is achieved when all of the biological indicators register zero growth on each of three consecutive runs.

3.3.1.7 Disinfectants

The only liquid chemical disinfectant that will be used initially with hemorrhagic fever (lipid enveloped) viruses will be MicroChem plus at a concentration of at least 5% (with a minimum of at least 10 min of contact time). Research staff will work with

EHS to confirm the efficacy of this disinfectant with their specific viruses, once we are able to bring them into NEIDL. Also, before other viruses are introduced the applicability of this disinfectant will be investigated and alternatives considered. In the event other disinfectants are indicated, these disinfectants will be assessed for efficacy before being allowed to be used routinely in the BSL-4.

3.3.2 Verification

3.3.2.1 Autoclave

Autoclave cycles are verified using chemical and biological indicators placed within a load of solid or semi-solid waste (laboratory waste, animal bedding, cages & bottles), or potentially contaminated reusable materials (e.g., scrubs and towels). Following an autoclave cycle, the chemical indicator is immediately examined to confirm that the desired cycle parameters were met. The biological indicator, usually self-contained vials containing *Geobacillus stearothermophilus* spores, is then separated from the load and processed by incubation at 55-60°C for 24 hours (using rapid readout methods – e.g., Prospore II self-contained indicators), or up to 7 days (in the case of regular spores and media) to verify efficacy of the decontamination cycle. The absence of growth indicates confirmation that the biological indicator spores were rendered non-viable and assurance that the waste/reusable items were sterilized. During incubation, the autoclaved load is isolated and stored until the cycle has been validated. Once confirmed as sterile, the verified biological waste is then disposed of by an approved method (e.g., treated as medical biological waste, boxed and shipped out for 3rd party disposal, or in the case of animal carcasses, subject to tissue digestion in-house using alkaline hydrolysis).

3.3.2.2 Space

Gaseous decontamination of spaces is verified using appropriate biological indicators applied throughout the suite or space(s). Once a validated gas or vapor method is established the same parameters are used for all space decontaminations and the efficacy verified each time using biological indicators. Spore strips in glassine envelopes or stainless steel coupons in Tyvek pouches are used. Typically, *Geobacillus stearothermophilus* spores at a concentration of more than 10^6 are used. Typically 30 locations will be selected at various heights and triplicate biological indicators are deployed at each location. After exposure the biological indicators and non-exposed biological indicator spores (controls) are processed as outlined above (see paragraph 3.3.1). The triplicate BIs at each location is to take account of uncertainty that can be

created by false positives (Krushefski, G. http://biologicalindicators.mesalabs.com/wp-content/uploads/sites/31/2014/03/Spore-News-Vol-9-No4.pdf). Hence, growth of 1 of the 3 BIs at a particular location would not be cause for concluding the decontamination to be unsuccessful. If 2or 3/3BIs grow in any location the decontamination process has failed and must be repeated. Up to two locations where 1/3 BI's show growth will be permitted, but no more. If greater than this, the decontamination process must be repeated, Hence, the criterion for verification of a successful decontamination can be summarized as: zero growth in 88/90 BIs (or 97.8%) with the two growth positive BIs present in different locations.

3.3.2.3 Filters

There are various types of filters and housings at NEIDL. These include the exhaust and supply HVAC HEPA filters, the plumbing vent filters, autoclave HEPA filters and numerous small filters (e.g., for O2 manifolds, APR doors, and room pressure sensors). In the event that HEPA filters fail to pass the integrity leak test (showing that particulates have the potential to pass through the filter) or if the filter becomes loaded, it must be decontaminated before it can be replaced. Decontamination cycles are first validated for all of the types of filters and housings (see above). The validated cycle is applied on each subsequent occasion but to verify continued successful decontamination, biological indicators are inserted into the return hose. These are retrieved, processed and read before the filter and housing can be released for maintenance.

3.3.2.4 Liquid Effluent Decontamination System

Each batch of liquid effluent that is processed in the Effluent Decontamination System is monitored for time, temperature and pressure. This batch data is then saved as a batch report that is checked immediately after each run and archived by EHS. The EDS will not discharge to the pH neutralization system if all of the programmed process parameters are not met. Routine verification of the efficacy of this decontamination system is accomplished on a quarterly basis for all 3 tanks (one tank being verified each month) by inserting three self-contained biological indicators into the "wet well" filled with thermal transfer oil that traverses diagonally through the tank (accessible through specific ports). The self-contained vials containing at least a 10^6 concentration of *Geobacillus stearothermophilus* spores in media, are retrieved and processed with non-treated controls at 55-60°C for 48h. There is a color change when growth occurs. So, successful decontamination is confirmed when all of the exposed indicators remain the same color, while the non-exposed control shows a color change.

3.3.2.5 Gamma irradiation (inactivation) The verification of inactivation of viable BSL-4 organisms will require a sample to be taken back into the laboratory and cultured. Only upon no growth being observed will the batch be considered effectively inactivated.

4.0 DECONTAMINATION APPLICATIONS

The following section provides a detailed list of laboratory items, materials, or incidents whose decontamination represents the maintenance of a critical biological safety containment barrier. Decontamination is performed as a daily routine laboratory practice and whenever shutdown for maintenance is required. The detailed waste disposal application for biohazardous wastes generated in the facility may be found in Section 17 Waste Disposal Plan.

4.1 Solid and Semi-solid Waste

As mentioned earlier, infectious or potentially infectious laboratory solid and semi-solid wastes undergo sterilization in a “pass-through” type autoclave chamber using steam heat treatment to destroy microorganisms. Waste is generated through regular laboratory and housekeeping activities, husbandry of live animals, and through necropsy procedures.

4.2 Supplies and Equipment

Laboratory supplies consist of small impermeable solid objects (such as pipettes, and laboratory plasticware) whose function in a laboratory is associated with the execution of a specific task. These materials are routinely exposed to biological hazards within BSL-4 laboratory spaces warranting their decontamination. The BSL-4 laboratories are currently set up to perform research upon hemorrhagic fever (lipid enveloped) viruses. All of these are susceptible to disinfection using the disinfectant, MicroChem Plus (a quaternary ammonium compound) at a minimum concentration of 5% and 10 minutes of contact time. MicroChem plus will be used exclusively in the BSL-4, until other types of viruses are introduced. Consideration of the applicability of this disinfectant or the need to change the disinfectant used will always be conducted prior to the introduction of novel viruses into the BSL-4. Typically ethanol would not be used as a disinfectant given that it is difficult to maintain the required contact time due to evaporation. However, 70% ethanol may be used to remove the sticky residue that can be left from the use of MicroChem. Sharp objects such as scalpels are not reused. They are disposed of in sharps containers which, in turn, are autoclaved out of the BSL-4 facility. Supplies that are not heat-sensitive can be removed from a BSL-4 laboratory by decontamination in an autoclave cycle.

Laboratory equipment consists of mechanical components and fixtures that assist workers in their research activities (i.e., biosafety cabinets, centrifuges). All equipment that is brought into the BSL-4 must be documented on a BSL-4 equipment entry form and a plan for decontamination of that equipment will be determined at that time. Equipment destined for repair or removal to a non-BSL-4 space is decontaminated first by surface decontamination in the BSL-4, then by gaseous vapor decontamination (using VHP or formaldehyde) in the fumigation airlock. Equipment with microfluidic tubing requires the tubing to be either flushed with disinfectant (in the BSL-4 laboratory) or the tubing discarded (via autoclaving) to be replaced later with new tubing. The equipment is partially dismantled in the airlock to aid in penetration of the gas/vapor into the inner compartments of the equipment and the efficacy of this decontamination is confirmed by use of biological indicators placed within the unit(s) before it is released from that space.

4.3 Positive Pressure Protective Suits

All laboratory personnel are required to exit the BSL-4 space through a chemical disinfectant shower. MicroChem plus at a concentration of 5% is used to decontaminate the protective suit. An atomizer continuously sprays the suit from 26 nozzles for three minutes, which is then followed by four minute water rinse. Following the chemical shower, personnel exit the shower room and enter the suit room and remove the decontaminated suit.

4.4 Biological Spills

In the case of a biological fluid spill, the spill is covered with absorbent material and then MicroChem plus at a concentration of 5%, is poured onto the absorbent material to thoroughly soak it, working from the outside towards the center. For routine follow up decontamination of any solid surface, such as room surfaces, equipment surfaces, or laboratory bench-tops, subjected to spills of contaminated fluids, further application of MicroChem plus would occur by spraying or wiping the disinfectant onto the surface. If a spill were to penetrate a difficult to access space, a gaseous or vaporous decontamination process will be used to decontaminate the entire space.

4.5 Air Stream

The BSL-4 air stream consists of supply, exhaust, and ambient air within the BSL-4 space. Supply air passes through a HEPA filter and exhaust air is filtered through two HEPA filters in series.

4.6 Animal Decontamination

See autoclaving of animal carcasses above (section 3.3.1.1).

4.7 Sample Decontamination

Any liquid samples of infectious agents are disinfected by applying MicroChem plus at a concentration of at least 5% within the BSL-4 laboratory (and leaving overnight before sink or drain disposal). The effluent is then passed to the EDS tank where it is sterilized by heat treatment before disposal to the pH neutralization system.

For transport of a viable sample outside of BSL-4 containment, samples in a primary and secondary container will be surface-decontaminated using 5% MicroChem plus. Samples contained in a leak-proof container will pass through a chemical "dunk tank" containing a chemical disinfectant solution (again, 5% MicroChem plus). From inside the BSL-4 laboratory space, the sample package is submerged completely and allowed to be disinfected. The samples are retrieved from the non-containment, "clean" side of the dunk tank accessible in the airlock. Following the SOP for removing viable materials, the sample(s) may be safely removed and either brought to the irradiator for final sterilization or packaged appropriately for transport to off-site collaborators.

4.8 Plumbing

The effluent piping that connects the BSL-4 sinks and drains to the EDS system consists of double walled stainless steel piping. The inner pipe acts as the normal conduit for the liquid effluent, and is surrounded by an outer pipe that will contain any leakage in the event that there is a breach of the inner pipe.

Prior to maintenance of BSL-4 plumbing lines under normal operations, the plumbing is isolated (by closing the valves proximal to the cook tanks) and the inner pipework filled with a chemical disinfectant that is effective against the agents used in the lab (initially, this will be 5% MicroChem plus). After the prescribed contact time (at least 10 minutes, but out of an abundance of precaution would be much longer in practice), the disinfectant will be drained into the effluent decontamination system .

In the event there is a breach of the inner piping (as determined by the leak detection system), the plumbing will be isolated and the pipework filled with MicroChem plus (as above). After the appropriate contact time (at least 10 minutes) this will then be drained from the inner pipe into the EDS tank. The outer pipe will then be drained by opening the appropriate valve. Once the pipe has been allowed to dry out, the outer pipe would be decontaminated using chlorine dioxide (to 720 ppm/hrs, or steam for at least 2h).

5.0 EMERGENCY RESPONSE APPLICATIONS

In the event of an emergency, the Incident Response Plan describes safeguards that are in place at the NEIDL to contain all high-risk organisms. These include a BU emergency response team that will be trained to handle all emergencies, including biological spills in BSL-4 laboratories. The team will coordinate their response with local emergency responders as part of a joint response. All BSL-4 laboratories and equipment that may have become contaminated or potentially contaminated during an emergency will be decontaminated using the facility and equipment decontamination process as described above. All emergency decontamination procedures will be a joint effort coordinated through EHS, Facilities Management & Planning, and the NEIDL emergency response team.

6.0 TRAINING

All EHS and biocontainment operations staff conducting the decontamination of facilities, equipment, and decontamination during emergencies will undergo training prior to working on any BSL-4 containment system. This decontamination training will be arranged to occur through outside providers e.g., Steris for VHP, B&V Testing for formaldehyde, and Clordisys for chlorine dioxide. Once EHS and biocontainment operations staff are trained and can be considered "experienced", these staff will provide additional training and oversight to newer, less experienced staff members. BSL-4 research staff will be trained on routine research practices as part of their required training. No member of the research team or support staff will handle any materials or enter the laboratory without participating in training addressing requirements for personal, equipment, facility, and emergency decontamination. Annual training requirements will include a discussion of key decontamination principles and procedures.

7.0 ANNUAL REVIEW

An annual review will be completed of all decontamination systems and standard operating procedures relating to these systems. All research team and support staff will be refresher-trained on any changes made to any procedure as part of annual training.

8.0 DEFINITIONS

Authorized Individual: "Authorized Individual" means a person who the DHHS Secretary or USDA Animal and Plant Health Inspection Service Administrator, and the BUMC Responsible Official have approved to have access for the possession and use of select agents at the NEIDL based on a Security Risk Assessment by the Attorney General.

Decontamination: "Decontamination" is described by Block (2001) as "disinfection or sterilization of infected articles to make them suitable for use" (p. 22).

Disinfection: "Disinfection" is described by McDonnell (2007) as the antimicrobial reduction of the number of viable microorganisms, or bioburden, on or in a product or surface to a level previously specified as appropriate for further handling or use (p3).

Equipment: Laboratory "equipment" consists of mechanical components and fixtures that assist researchers with complex functions. Examples of equipment include: biological safety cabinets, tabletop or floor model centrifuges, and incubators.

Principal Investigator: "Principal Investigator" means an authorized individual who is approved by the Responsible Official (RO) to direct a select agent or toxin project or program and who is responsible for the scientific and technical direction of that project or program.

Risk Assessment: "Risk Assessment" is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material (such as a toxin), the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a laboratory-acquired infection (LAI), and the probable consequences of such an infection. The information identified by a risk assessment provides guidance for the selection of an appropriate biosafety level(s) and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Responsible Official: The CDC Select Agent "Responsible Official" (RO) is an authorized individual with responsibility, authority, and control to ensure compliance with the DHHS and USDA Rules and Regulations pertaining to the possession, use, and transfer of select agents and toxins.

Select Agent: "Select Agent" means a biological agent or toxin that has the potential to pose a severe threat to public health and safety. The DHHS Secretary and the USDA Animal and Plant Health Inspection Service Administrator have responsibility for determining select agents. Select agents are listed in 42 CFR Part 73 and 9 CFR Part 121.

Sterilization: "Sterilization" is "the act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms" (Block, p. 21).

Supplies: "Supplies" is the term used in this *Decontamination Plan* to mean small impenetrable solid objects whose function in a laboratory is associated with the execution of a specific task. Such examples include pipettes, vortex, and rotors.

Validation: Validation is a rigorous process by which the various decontamination methods and cycles are established as producing at least a 6 log reduction of resistant microbiological materials (e.g., bacterial test spores), or meeting other agreed upon criteria. Typically there are two steps: a temperature mapping (e.g., to ensure uniform heating), and then microbiological challenge using indicators such as *Geobacillus stearothermophilus* spores. The NEIDL requirement is for 100% kill on each of three consecutive decontamination runs. Typically validation processes only need to be repeated if there are major repairs to equipment or significant changes within the building (e.g., large changes in supplied steam pressure).

Verification: Verification is the continued confirmation on a scheduled basis that the decontamination process or cycle remains effective as originally validated. Indeed, successful verification confirms that a specific decontamination process has destroyed microorganisms within the waste biological material(s)

9.0 KEY REFERENCES AND RESOURCES

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