# Kennesaw State University
# Biological Safety Manual

# Working with Biologically Hazardous Materials in the Lab

## Common Acronyms

**A.** KSU – Kennesaw State University
**B.** USG – University System of Georgia
**C.** IBC – Institutional Biosafety Committee
**D.** IACUC – Institutional Animal Care and Use Committee
**E.** EHS – Department of Environmental Health and Safety
**F.** BSO – Biosafety Officer
**G.** PI – Principal Investigator
**H.** NIH – National Institutes of Health
**I.** OBA – NIH Office of Biotechnology Activities
**J.** ABSA – American Biological Safety Association
**K.** NASBB – National Advisory Board on Biosecurity
**L.** DURC – Dual Use Research of Concern
**M.** OSHA – Occupational Safety and Health Administration
**N.** OCGA – Official Code of Georgia
**O.** GAEPD – Georgia Environmental Protection Division
**P.** EPA – Environmental Protection Agency
**Q.** USDA – United States Department of Agriculture
**R.** APHIS – Animal and Plant Health Inspection Service
**S.** CFR – Code of Federal Regulations
**T.** CDC – Centers for Disease Control
**U.** BMBL – Biosafety in Medical and Biomedical Laboratories
**V.** RG – Risk Group
**W.** BSL – Biosafety Level
**X.** ABSL – Animal Biosafety Level
**Y.** BBP – Bloodborne Pathogens
**Z.** OPIM – Other Potentially Infectious Materials
**AA.** LAI – Laboratory Acquired Illness
**BB.** BSC – Biosafety Cabinet
**CC.** SOP – Standard Operating Procedure
**DD.** IPM – Integrated Pest Management

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# 1. Purpose and Scope

## A. Purpose

This document will focus on the regulatory requirements and best practices for the safe handling and manipulation of biological hazards encountered when working in research laboratories in KSU facilities. The information provided is based upon the National Institutes of Health (NIH) Guidelines for Research

/TJ BDC LBody with Recombinant DNA Molecules, the Guidelines, and KSU-Td[le)10..17)-3.

- Collectively, the membership shall have experience and expertise in biohazardous materials and recombinant DNA technology.
- The membership shall have the capability to assess the safety of biohazardous and recombinant DNA research and identify any potential risks to public health or the environment.

The IBC shall include at least one individual with expertise in microbial pathogens and rDNA containment principles. The IBC shall have at least two members unaffiliated with the institution (apart from their membership on the committee) and they shall represent the interest of the surrounding community with respect to public health and protection of the environment.

All appointments to the committee shall be for three-year terms. Members shall be appointed, or reappointed, by the Vice President of Research or designee. The committee's current membership may be obtained by contacting the Office of Research at (470) 578-6738. Additional duties regarding the IBC are outlined in the IBC Charter and Procedures Manual.

Under the KSU Biosafety Program, the IBC is responsible for:

- Local oversight and review of activities that use the following potentially biohazardous materials or procedures:
    - Recombinant or synthetic nucleic acid molecules (rDNA/RNA).
    - Select agents and toxins, as determined by the Federal Select Agent Program (approval of research involving these activities is contingent upon their approval and registration with the Federal select agent program – see "Other Regulatory RequireTf0.001 Tc -0.0 P(.005 T4o)-5

- Providing guidance with regard to laboratory design in accordance with USG laboratory design guidelines.
- Reporting to the IBC and the institution any significant problems, violations of the NIH Guidelines, and/or any significant research

- Ensuring that lab personnel demonstrate proficiency in standard and special microbiological practices before working with biohazardous agents.
- In conjunction with the BSO, ensuring that physical containment systems, support equipment, waste disposal and operation of lab are in accordance with the design and safety guidelines of the BSL-2 facility.
- Performing regular checks/assessments of containment devices, equipment, and PPE.
- Notifying the BSO and/or EHS of any incident or problem that compromises the safety of the staff or the integrity of the lab.

## H. Employees Working in the Lab

All laboratory personnel who engage in research activities have a shared responsibility for ensuring their own safety while working in the lab. Responsibilities for all laboratory personnel include, but are not limited to:

- Completing all assigned EHS and lab specific training to work proficiently and independently in the laboratory.
- Conducting all research activities in accordance with University's policies, procedures, and guidelines, including the standards in this Biological Safety Manual.
- Being familiar with the standard operating procedures for laboratory activities, the potential hazards of the infectious agents in use, and emergency procedures.
- Employing good housekeeping practices to help maintain the laboratory in good working condition.
- Completing all medical surveillance requirements.
- Reporting any medical restrictions, reportable illnesses, and any event that may be the result of an exposure to the PI and Lab Manager/Supervisor.
- Reporting irregular laboratory conditions or accidents to the PI and EHS immediately.
- Performing all duties as assigned in a prudent manner.

## I. Students in the Laboratory

Students who are participating in laboratory activities for undergraduate academic credit or volunteer credit must be supervised by a PI, lab manager, or teaching assistant while in the lab, especially when working with biohazards. Student activities involving hazardous materials, equipment, and/or processes should be limited at the discretion of the PI/Lab Manager. Students have a shared responsibility for ensuring their own safety while participating in lab activities. These responsibilities include, but are not limited to:

- Completing and submitting the KSU Volunteer Program form prior to beginning laboratory activities (See Appendix A).

***Note:*** 9m001 Tw2.64 (9m)y

- Being familiar with the SOPs for laboratory activities, the potential hazards of the materials in use, and emergency procedures.
- Employing good housekeeping practices to help maintain the laboratory in good working condition.
- Reporting any injuries, reportable illnesses, and any event that may be the result of an exposure to the PI and Lab Manager/Supervisor.
- Reporting irregular laboratory conditions or accidents to the PI and EHS immediately.
- Performing all duties as assigned in a prudent manner.

## 3. Regulatory Requirements

The biosafety practices outlined in this manual are based on the NIH Guidelines and the best practices found in the BMBL. There are additional guidance documents and regulations imposed by various funding agencies that individual PIs must be aware of and incorporate into their lab-specific Biosafety Manuals. Biosafety requirements must be followed to ensure the continuation of grant funding from federal agencies

# 7. Other Regulatory Requirement

- Assessing the hazard – What could potentially go wr

### 2. Risk Group 2

Agents are associated with human disease that is rarely serious, and for which preventive or therapeutic interventions are often available. Examples: *E. coli* O157:H7, *Salmonella typhimurium*, and *Cryptosporidium parvum*.

### 3. Risk Group 3

Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Examples: *Yersinia pestis*, *Brucella abortus*, *Mycobacterium tuberculosis*.

### 4. Risk Group 4

Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Examples: Ebola virus, Macacine herpesvirus (formerly Cercopithecine herpesvirus 1, also called Herpes B or Monkey B virus).

Microorganisms that are RG1 require standard laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities. Many of the agents likely to be handled experimentally at KSU are RG1, RG2 or RG3 pathogens (low, moderate and high hazard, respectively). RG3 agents typically require more sophisticated engineering controls than standard laboratories, as well as special handling and decontamination procedures.

Microorganisms classified as RG2 or higher have been reported to cause infection and disease in otherwise healthy adults. Many have been associated with laboratory-acquired infections. The progression from exposure, to infection, to disease following contact with an infectious agent depends upon the route of transmission, inoculum, characteristics of the agent, and resistance of the person exposed, whether innate or acquired. Not all exposures result in infection, and even fewer develop into clinical disease. However, it is prudent to handle such agents at the prescribed biosafety level.

## 10. Routes of Infection and Exposure Sources

Depending on the organism in question, pathogens can be transmitted via four potential routes of exposure. The most common routes of exposure are:

- Inhalation – breathing in airborne materials such as infectious aerosols or dusts)
- Absorption (exposure of mucous membranes to infectious droplets)
- Ingestion (from contaminated hands or utensils)
- Percutaneous inoculation (injection, incision, or animal bite).

Appropriate precautions can be implemented to reduce the risk of such exposures.

### A. Clinical and Pathological Specimens

Specimens from human patients or animals may contain infectious agents. Specimens most likely to harbor such microorganisms include blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, and tissues.

infectious materials should practice general universal precautions – an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C (HCV) and other bloodborne pathogens. Such personnel are required by law (OSHA 29 CFR 1910.1030) to undergo BBP training. OSU, this training may be satisfied either online or .8 (s)-Polyvinyl Alcohol (PVA) (w6 (a)3.tg652

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excreta can also be a source of infectious microorganisms, investigators should take precautions to

## F. Research Laboratories

The risk of exposure increases with experiments in research laboratories using high concentrations or large quantities of pathogens.

- Use an appropriate disinfectant to decontaminate surfaces (e.g. – 10% bleach solution, 70% ethanol, etc.)
-

The KSU biosafety manual must be available and accessible to all laboratory employees. A lab specific biosafety manual, or at minimum, biosafety SOPs should be prepared and available to all employees.

The collection and storage of serum samples from at-risk personnel should be considered.

Potentially infectious materials must be placed in durable, leak-proof containers when collecting, handling, processing, storing, or transporting them within a facility.

Lab equipment must be decontaminated by properly trained personnel using an appropriate disinfectant both regularly and after all potential contamination.

All equipment used with infectious materials must be decontaminated prior to repair, maintenance, or removal from the laboratory.

## 8. Safety Equipment and Personal Protective Equipment

Biosafety cabinets, PPE, or other physical containment devices must be used as follows:

- Anytime procedures are conducted that have the potential for creating infectious aerosols or splashes (e.g. – pipetting, centrifuging, grinding, blending, mixing, shaking,

Contaminated protective clothing must be either disposed of as biohazard waste, or laundered by an institutional laundry service (i.e. - laboratory protective clothing must not be taken home for laundering).

When contaminated, eye and face protection must either be discarded as contaminated waste or decontaminated prior to reuse.

### *a. Procedure for Laundering Laboratory Protective Clothing*

Disposable protective clothing, which is placed in biomedical waste after use, is preferred. Reusable laboratory protective clothing must be decontaminated by autoclaving before disposal or laundering. Reusable laboratory protective clothing must be bagged, autoclaved and then washed before being returned to the laboratories for reuse.

## 9. BSL-2 Facility Requirements

A BSL-2 facility must have the following safeguards in place:

- Laboratory doors should be self-closing and equipped with a locking mechanism.
- In addition to having hand washing sinks, the sinks should be hands free (e.g. - foot petal operated) or motion operated to avoid touching. There should also be sinks located near exit doors.
- All laboratory equipment must be [illegible]

For additional information on biosafety containment in rDNA experiments, refer to the NIH Guidelines.

# I. Biosafety Cabinets

## 1. BSC Use Protocol

A properly certified and operated BSC in combination with appropriate contamination control procedures and aseptic techniques will do an excellent job of controlling airborne contaminants. They are equipped with High Efficiency Particulate Air (HEPA) filters that remove contaminants from the air. BSCs are designed to provide 3 levels of protection:

- Personnel or operator protection
- Product or sample protection
- Environmental Protection

For example, in a Class II AII BSC, approximately 70 percent of the intake air is recirculated inside the cabinet, while 30 percent is exhausted through the exhaust port in the top of the cabinet back into the laboratory. All filtered air is considered free of contaminants as long as the BSC and its components are working properly. Additional information on biosafety cabinets, the different types of BSCs, and how they function can be found at http://www.nuaire.com/products/biological-safety-cabinets/how-class-two-bsc-work.html.

BSCs are to be used only with biological materials, unless otherwise approved by EHS, and are not to be confused with Chemical Fume Hoods, which provide only personnel or operator protection.

- Wa[illegible] hands thoroughly before and aft[illegible] ki[illegible] our BSC. Weari[illegible] lab[illegible] oat and gloves while working [illegible] in[illegible] s your safety a[illegible] duce cont[illegible] of research [illegible]als.
- On a [illegible] is decon[illegible]te [illegible] work gril[illegible] work surface[illegible] parts are removal[illegible]
- cou.1826

- When inserting your arms into the BSC, remember that they are penetrating a delicate "curtain" of air that can only provide protection from contamination as long as it is not disrupted. Allow the air curtain to stabilize around your arms before starting work.
- Avoid making rapid, jerking arm motions. Use smooth motions that do not disrupt the air curtain.
- If you need to introduce new items or remove items from the BSC, move your arms in and out slowly to minimize airflow disruption.
- If you use equipment that creates air turbulence, i.e., centrifuge, blender, or sonicator, place the equipment as far back in the BSC as possible (usually 1/3 of the way back from the front intake grill is acceptable). Stop other work while the equipment is operating.
- Follow good microbiological techniques (e.g. - holding open tubes and bottles as horizontal as possible.
- Never pipette materials by mouth. Use mechanical pipetting aids.
- Keep your hands away from your face (face protection helps to minimize the potential for this route of exposure).
- Do

- **Bags:** Bags have the potential for creating aerosols when moved. Seal autoclave bags within the cabinet and place within a second bag. Carefully add water to the primary bag before sealing (25 ml for smaller bags, 200 ml for larger bags). The addition of water will help to generate steam within the bag during the autoclave cycle.

- An externally mounted drain valve is located under the drain pan of many BSC's. In the event of an accidental spill, you can remove large volumes of disinfected, spilled materials from under the work surface. The drain valve is not to be connected to a sink or similar drain. A bucket can be used to collect any spilled liquid for decontamination and disposal.

### 3. Cleaning the BSC

- After completion of work, enclose or cover all equipment and materials.
- Wipe items down with an appropriate disinfectant prior to removal from the BSC.
- Allow the BSC to run for five minutes to purge airborne contaminants from the work area.
- Decontaminate interior surfaces (work surface, grilles, sides, back and inside front view screen) with an appropriate disinfectant after removal of all materials, cultures and

- Reaching into sharps disposal containers to retrieve a sharp object

## 2. Sharps Disposal

- Accessing a vein or artery;
- Administration of medications or fluids; and
- Any other procedure

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## C. Autoclave Use

Steam sterilization is the most reliable means for complete destruction of all microbial life, including bacterial spores. Autoclaves accomplish this by generating moist, high temperature, pressurized steam

***CAUTION: Opening the autoclave door too quickly may result in glassware breakage and/or steam burns on your skin. The decontaminated materials should be allowed to cool for 10 minutes before they are taken out of the autoclave.***

### *a. Things to Avoid:*

- Dead air pockets where steam cannot penetrate (i.e., closed screw cap tubes) because temperature within the air pocket is much lower than the saturated steam.
- Avoid dry packages; add some water to the load. To avoid creation of infectious aerosols while adding water, trickle water down the sides of the container instead of pouring water directly onto the material in the container.

### *b. Autoclave Processing Times*

The US Environmental Protection Agency (EPA) has reported that "Infectious wastes from departments of health care facilities may be rendered noninfectious by subjecting the waste to autoclave temperatures of 121°C (250°F) and 15 minutes of pre-vacuum of 15 psi for the following dwell times when proper containers are used:"

EPA Recommended Decontamination Processing (Dwell) Time

| ITEM | DWELL TIME |
|---|---|
| **TRASH** | 60 Minutes |
| **GLASSWARE** | 60 Minutes |
| **LIQUIDS** | |

vial should remain clear without evidence of turbidity (no growth) and the pH should not change. If the autoclaved biological indicator is turbid (cloudy, indicating growth) the autoclave did not function properly. Notify your area supervisor when this happens.

### 2. Chemical Color Change Indicators

Chemical indicators for steam autoclaves change color after being exposed to normal autoclave operating temperatures of 121°C (250°F) at 15 psi. Hence, chemical indicators can give you a quick visual reference for heat penetration inside the load. Chemical indicators should be positioned near the center of each load, and toward the bottom front of the autoclave.

***CAUTION: Most chemical indicators can only be used to verify that your autoclave reaches normal operating temperature for decontamination, 121°C (250°F). Chemical indicators alone are not designed nor intended to prove that organisms are actually killed during a decontamination cycle.***

### 3. Tape Indicators

Tape indicators are adhesive backed paper tape with heat sensitive, chemical indicator markings. Commonly used heat sensitive markings include diagonal stripes (autoclave tape), and/or the word "sterile". These markings only appear when the tape has been exposed for a few minutes to normal autoclave decontamination temperatures. Tape indicators should be used on all material decontaminated by autoclaving to show that the material has been processed. A three to four inch strip of autoclave tape placed on the outside of the autoclave pan, bag, or individual container is sufficient. If the temperature sensitive tape does not indicate a temperature of at least 250° F (121° C) was reached during the sterilization process, the biomedical waste is not considered decontaminated.

***CAUTION: Tape indicators can only be used to verify that the autoclave is reaching normal operating temperatures for decontamination, 121°C (250°F). Tape indicators alone are not designed nor intended to prove that organisms have actually been killed.***

### 4. Odor Control

Some waste materials, such as anaerobic bacteria, feces or decaying organic materials have an extremely noxious odor. When decontaminating these materials, it may become necessary to add an odor control additive to the load. A scoop full of unused animal bedding (cedar shavings) works quite well. These additives may produce chemical exposure symptoms for some people.

### 5. Training

It is imperative that supervisors provide training to qualify their staff for both the basic use of autoclaves, as well as to decontaminate materials. Qualified autoclave users should understand the time, temperature, and pressure relationships required for proper decontamination of infectious materials. Additional training on handling materials to be decontaminated should also be provided. Supervisors should maintain permanent records of training provided to their staff. EHS should also receive copies of the training records. Additional training support on the effective use of autoclaves is available from the Biosafety Officer.

## 2. Bloodborne Pathogens Training

Employees who have the potential for occupational exposure to bloodborne pathogens (BBPs) or other potentially infectious materials (OPIM) must complete BBP training in accordance with OSHA 29 C2 (S.6 (0F)4 ( OR.7 ( O)1.7 ( C0.7 ( C1.7 (t)01.5 (9).11.5 (t)017 (t)3.7 ( C0TJEMC /LEM/Spa

- Hospitalization or medical treatment (beyond first-aid) for both KSU's and non-KSU personnel.
- Fire
- Property damage exceeding $1,000.00.

- All other incidents must be reported in writing within 24 hours of becoming aware of the incident, injury, or illness.

## C. Post-Exposure Evaluation and Follow-Up

Post-exposure evaluation and follow-up will be provided at no cost to employees following exposure incidents. The post-exposure monitoring periods are dependent on the type of exposure and the variety of incubation periods characteristic of the infectious agents.

All employees who have an exposure incident will be offered a confidential post-exposure medical evaluation and follow-up appointment. The post-exposure medical evaluation and follow-up include the following: -rJ0.00d(-)Tj.62 0 Te(-)80. .62 0 Tr2 (s80.4(-)Tj(-)Tj.61)12.9me(-)80. 0 T326 0w 5 (0)-4.6

Other potential items to include in the kit are as follows:

- Disposable Tyvek suit
- Sponges
- Autoclave bags
- Kevlar gloves and sleeve protectors
- N-95 respirator

## D. Spills in the Biosafety Cabinet

- Allow the BSC to continue running.
- Stop all work and secure containers and samples.
- Don personal protective equipment (i.e. – goggles, double gloves, lab coat/apron) if not already wearing it.
- For a s

- Decontaminate the w

- Apply a

# 17. Medical Surveillance Program

## A. Health Status

It is a prudent practice to inform your personal physician about your occupational risks, especially work with biohazardous or potentially biohazardous agents, so the information can be on record. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include, but are not limited to: diabetes, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy, even if only temporary.

## B. Medical Surveillance

A medical surveillance program of KSU personnel engaged in biological research should be conducted by a University-contracted physician. The program may include precautionary measures including, but not limited to, immunizations, periodic physical examinations, and collection of serum samples.

The purpose of the medical surveillance program is to:

a) Ensure individual employees are physically fit for the nature and extent of the work to be undertaken.
b) Recommend appropriate medical precautions.
c) Perform periodic reassessment of employees to determine if medical conditions associated with employment are prevalent and, if so, to undertake definitive measures to alleviate them.

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

a) Nature of the research project in which involved
b) Biological agents to which directly or potentially exposed
c) Factors relating to the current or previous health status of the individual.

The EHS is to provide the University-contracted physician with guidelines and descriptions of conditions that may have significance for personnel assigned to laboratories. It is the ultimate respons8ths5senr 1-1./p(le)-1.1.ow0 T Tc ehe Us8r5v

## D. Restrictions

### 1. Pregnancy

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Women that are pregnant or become pregnant are encouraged to inform their supervisors or PIs and their physician. Employees are urged to discuss exposure issues with their supervisors or PIs regarding associated risks of research being conducted and pregnancy. Your personal or University-contracted physician should be consulted to give advice on necessary precautions.

### 2. Other Restrictions

Restrictions or recommendations will be made on an individual basis after discussion with the employee's personal physician. Examples of conditions that may warrant special precautions are HIV infection, immunosuppressive conditions or drug therapy that suppresses the immune system. Therefore, if you have any of the above conditions, you must inform your physician.

# 18. Laboratory Design

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. Laboratory design features for BSL-1 and BSL-2 facilities can be found in Appendix A and in the table below.

**SUMMARY OF BIOSAFETY LEVEL REQUIREMENTS**

| **Biosafety Level** | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Isolation of laboratory** | No | No | Desirable | Yes |
| **Room sealable for decontamination** | No | No | Desirable | Yes |
| **Inward air flow ventilation** | No | Desirable | Yes | Yes |
| **Mechanical ventilation via building system** | No | Desirable | Yes | No |
| **Mechanical, independent ventilation** | No | No | Desirable | Yes |
| **Filtered air exhaust** | No | No | Desirable | Yes |
| **Double-door** | No | No | Yes | Yes |

## 19. Equipment Repairs and Service

Contaminated equipment that has to be sent out for repair/service or to be discarded must be

the best method(s) of containment. BMBL, Section V

Full Name of Volunteer: _____

Volunteer's Responsible Supervisor:_____

Volunteer Services: From: _____ To: _____

Purpose for Volunteer Services:

_____
_____
_____

Scope of Volunteer's Work and Duties (per responsible supervisor):

_____
_____
_____

Department/Director Approval: _____

Date Approved: _____ Email & Phone: _____

Responsible Supervisor Signature: _____

Volunteer Signature: _____

Parent's Signature (if under 18): _____

Please maintain copies of the *Agreement for Volunteer Service* and *Volunteer Services Description* forms on file with your Department/Unit for at least 2 yrs. Forward original copies to the Risk Manager, Janet Nash at jhull@kennesaw.edu or Mail Drop 1402.

**AGREEMENT FOR VOLUNTEER SERVICES**

I, _____, agree to work as a volunteer in

Volunteer's Signature Date

Parent's Signature (If volunteer is a minor) Date

## Appendix B

Waiver and Release Agreement regarding the Use

of Kennesaw State University Science Labs

**PLEASE READ THE FOLLOWING CAREFULLY BEFORE SIGNING:**

**LIABILITY RELEASE, INDEMNITY AND PROMISE NOT TO SUE:**

I, the undersigned below, in consideration of Kennesaw State University allowing my and/or my

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This Waiver and Release Form shall be governed by the laws of the State of Georgia, and any legal action related to or arising out of this Waiver and Release Form shall be commenced exclusively in the Superior Court in and for Cobb County, Georgia.

If I am executing this Waiver and Release Agreement on behalf of my child or ward, I certify that the information set forth above pertaining to my child or ward is true and complete. If I am executing this Waiver and Release Agreement on my own behalf, I certify that I am eighteen (18) years of age or older.

**I HAVE READ, UNDERSTOOD AND ACCEPT THE CONDITIONS OF THIS LIABILITY RELEASE, INDEMNITY, AND PROMISE NOT TO SUE.**

**Participant Information:** ***(Please PRINT)***

**Name:** _Rf-0.00 (Rf-0.00 (Rf-0.00 Rf-0.00 Rf-0.00  Rf-0.00  Rf-0.00  Rf-0.00) )Ɜ (S)1 ( L)-0.00  Rf-0.00Tc 0 T0n(