BIOSAFETY REGISTRATION FORM FOR BIOSAFETY LEVEL 2 AGENTS

Project Title: Microbiology Laboratory- Teaching Lab

BSL-2 Agent Used: *Bacillus cereus, Citrobacter freundii, Enterbacter aerogenes, Enterobacter cloacae, E. coli, Klebsiella pneumoniae, Micrococcus luteus, Micrococcus roseus, Mycobacterium smegmatis, Proteus vulgaris, Proteus mirabilis, Proteus morganii, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella typhosa, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Staphylococcus aureus, Entercoccus faecalis* (source used for identification of Biosafety Level 2 –Public Health Agency of Canada MSDS of infectious substances.)

Lab Principal Investigator: Kathleen Ferris (Director of Microbiology Teaching Laboratory)

Department: Division of Natural Sciences, Biology Department

Building & Room: Smith 303

Prepared By: Kathleen Ferris

PI Approval Signature: _____

Initial Approval Date: _____

Annual Registration Form Reviews

Reviewer	Annual Review Date

When should this Biosafety Registration Form be completed?

Biosafety level 2 (BSL-2) laboratories are required to prepare a laboratory-specific biosafety manual. A Biosafety Registration Form should be completed for each BSL-2 agent used. Each laboratory should maintain a copy of their registration forms with their hard copy of the University Biosafety Manual. The addition of these registration forms to the general University Biosafety Manual creates a lab-specific manual.

What are the benefits of completing a Biosafety Registration Form?

1. They are reviewed by the Biosafety Committee (IBC), which is composed of peer researchers with expertise to ensure biosafety considerations have been addressed.
2. They serve as written reminders to the staff of the correct way of conducting a procedure.
3. They should be used as training tools for all new staff. A record should be maintained to indicate which registration forms have been read and understood by the employee. This same record can be used to document the date an employee is competent to perform the procedure as outlined in the registration form.
4. Completing a Biosafety Registration Form forces the author to think through the procedure step by step and to modify those procedures if necessary.
5. The use of written Registration Forms allows your lab to ensure each researcher utilizes the appropriate work practices, safety equipment, and laboratory facilities to reduce the risk of exposures, while also maintaining compliance with applicable university policies, and external regulations and guidelines.

What are the primary considerations when completing a Biosafety Registration Form?

The registration form responses should be succinct to assure that they will be read and frequently referenced. The individual(s) who actually conduct the procedure on a daily basis should be the ones to complete the registration form since they are most familiar with the procedure. The PI should review and approve the registration form before it is implemented and the procedures are performed by other researchers in the lab.

How should the lab distribute and archive a Biosafety Registration Form?

A copy of the registration form should be available at all work stations where the BSL-2 agent is used, not in a file cabinet or on a shelf in the supervisor's office. The registration forms are working documents and must be available to your staff for reference and use. Each person working with the BSL-2 agent should be familiar with the applicable biosafety registration form. A copy of each form should also be maintained with the other laboratory biosafety resources.

BIOSAFETY REGISTRATION FORM
FOR WORK INVOLVING BIOSAFETY LEVEL 2 AGENTS

Please answer the following questions related to your research involving work with biosafety level 2 agents. Several questions include additional information to provide guidance on recommended biosafety practices.

1. Experimental Procedures:

Provide a summary of the nature and purpose of the research involving the BSL-2 agent. The purpose of the bacteria listed below is for teaching purposes. Bacteria are used to teach staining and isolation/enumeration techniques, antibiotic, antiseptic, and disinfectant sensitivity, and biochemical analysis for an identification process.
Describe the experimental procedures and techniques to be utilized in the project. Standard microbiological practices for staining and isolation/enumeration techniques, antibiotic, antiseptic, and disinfectant sensitivity testing, and biochemical analysis.

2. BSL-2 Agent Description:

Agent Description:		
	X	Bacteria
		Virus
		Fungi
		Parasite
		Rickettsial
		Other (describe): _____
Name of agent (genus and species): ***Bacillus cereus, Citrobacter freundii, Enterbacter aerogenes, Enterobacter cloacae, E. coli, Klebsiella pneumoniae, Micrococcus luteus,***		

***Micrococcus roseus, Mycobacterium smegmatis, Proteus vulgaris, Proteus mirabilis, Proteus morganii, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella typhosa, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Staphylococcus aureus, Entercoccus faecalis* (source used for identification of Biosafety Level 2 –Public Health Agency of Canada MSDS of infectious substances.)**

Strain of agent: Bacterial specimens were obtained from Carolina Biological Supply Company.

Ability to cause disease in humans (signs & symptoms of exposure):

Bacillus cereus- Opportunistic pathogen; intoxication characterized by two forms: an emetic form with severe nausea and vomiting and a diarrheal form with abdominal cramps and diarrhea; both forms are usually mild and self-limiting (24 hrs); immunocompromised individuals are susceptible to bacteremia, endocarditis, meningitis, pneumonia; also associated with posttraumatic endophthalmitis (ocular infection - rare).

Citrobacter freundii- pathogen, associated with nosocomial infections; causes diarrhea and secondary infections in immunocompromised patients and occasionally severe primary septicemia; *C. koseri* is associated with meningitis in infants <2 months old.

Enterobacter aerogenes, cloacae- Associated with a variety of infections including those of nosocomial origin; urinary, pulmonary, wound and bloodstream infections; often as a secondary or opportunistic infection.

E.coli- Self-limiting cholera-like disease in infants and adults; profuse watery diarrhea without blood or mucous; abdominal cramping, vomiting, acidosis, prostration, malaise and dehydration can occur; fever may or may not be present; symptoms usually lasts fewer than 5 days.

***Klebsiella* pneumoniae-**Frequent cause of nosocomial urinary and pulmonary infections; wound infections; secondary infection in lungs of patients with chronic pulmonary disease; enteric pathogenicity (enterotoxin); ozena (atrophy of nasal mucosa) and rhinoscleroma.

Micrococcus luteus, roseus- Most strains are saprophytic and non-pathogenic found in soil, water, dust, and dairy products; frequently found on the skin of man and other animals; some species are opportunistic pathogens (pathogenicity is uncertain because isolations are very frequently from sites where other potential pathogens are present); may occasionally participate in infections like endocarditis; localized cutaneous infections in the immune-compromised.

Mycobacterium smegmatis-Pulmonary disease resembling tuberculosis may be associated with *M. kansasii, M. avium-intracellulare*; lymphadenitis may be associated with *M. scrofulaceum, M. avium* complex; skin ulcers and soft tissue wound infections may be associated with *M. fortuitum, M. cheloni, M. ulcerans* and *M. marinun.*

Proteus mirabilis, morganii,vulgaris- Chronic urinary tract infections, bacteremia, pneumonia and focal lesions in debilitated patients or those receiving intravenous infusions, neonatal meningoencephalitis, empyema, osteomyelitis, cystitis, pyelonephritis, prostatitis.

Pseudomonas aeruginosa, fluorescens-Opportunistic pathogen, greatest risk of disease in the immunocompromised; most medical conditions arise from colonization of pathogen in the respiratory and urinary tracts or due to deep disseminated infections leading to pneumonia and bacteremia; chronic respiratory infections among cystic fibrosis patients; eye infections (especially in contact lens wearers); nosocomial infections causing severe and often fatal infections (case fatality in susceptible populations is 30%), increasingly associated with bacterial meningitis, abscesses, endocarditis.

Salmonella typhosa, typhimurium-Generalized systemic enteric fever, headache, malaise, anorexia, enlarged spleen, and constipation followed by more severe abdominal symptoms; rose spots on trunk in 25% of Caucasian patients; complications include ulceration of Peyer's patches in ileum, can produce hemorrhage or perforation; Common enterocolitis may result without enteric fever; characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration may result; case fatality of 16% reduced to 1% with antibiotic therapy; mild and atypical infections occur.

Serratia marcescens-Opportunistic infections of the endocardium, eyes, blood, wounds, urinary and respiratory tracts; infections are often severe or fatal; Notorious nosocomial pathogens, particularly *S. marcescens* which is responsible for 4% of hospital acquired pneumonias; Serious reactions have occurred to blood transfusions contaminated with *S. liquefaciens*; causes ocular complications associated with contact lenses.

Shigella flexneri-Acute disease of large and small intestine; diarrhea, fever, nausea, and sometimes toxemia, vomiting, cramps and tenesmus; stools contain blood, mucus and pus; alterations in consciousness may occur; mild and asymptomatic infections occur; severity of illness depends on host, dose and serotype - *S. dysenteriae* infections have up to 20% case fatality rate in hospitalized patients, while *S. sonnei* infections have negligible fatality rate; *S.flexneri* precipitate reactive arthritis (Reiter's syndrome) in some patients.

Staphylococcus aureus-Opportunistic pathogen, normal flora; produces a variety of syndromes with a range of clinical manifestations; clinically different in general community, newborns, menstruating women, and hospitalized patients; food intoxication is characterized by abrupt/violent onset, severe nausea, cramps, vomiting, and diarrhea using lasting 1-2days; animal bites can result in localized infections; may cause surface or deep/system infections in both community and hospital settings; surface infections include impetigo, folliculitis, abscesses, boils, infected lacerations; deep infections include endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis; systemic infection may cause fever, headache malaise, myalgia; newborns are susceptible to scalded skin syndrome (SSS) caused by exfoliative toxins; my be colonized during delivery resulting in sepsis meningitis; toxic shock syndrome is an acute multi-system illness caused by TSST-1 a super antigen; characterized by sudden onset, high fever, vomiting, profuse watery diarrhea, myalgia, hypotension erythematous rash.

Enterococcus faecalis- Normal inhabitant of intestinal tract (10^5-10^8 CFU's per gram of stool) and female genital tract; occasionally associated with urinary tract infection, bacteremia and bacterial endocarditis.

3. Risks/Hazards:

Identify "critical steps" in the procedure that may create a risk of exposure during sample preparation and/or experimental manipulations.

No critical steps in the procedures may create a risk of exposure during sample preparation and/or experimental manipulations.

Describe the potential routes of exposure that may be encountered. These may include inhalation of aerosols, needle stick or sharps cut, mucous membrane exposure from a

splash/droplet, or ingestion.

Potential routes of exposure are from mucous membrane exposure from a splash/droplet, or ingestion.

4. Engineering Controls:

Describe any safety equipment that may be utilized during the procedure. For instance, procedures with a potential for creating infectious aerosols (i.e. pipetting, grinding, blending, mixing, sonicating, etc.) will be conducted inside a biological safety cabinet.

The laboratory is posted as a Biosafety Level 2 outside of the laboratory with all doors permitted entrance to the lab limited to appropriate students and staff. The laboratory is a negative pressure lab with snorkel hoods available at the rear of the lab. A biological safety cabinet is available for use during culture transfers.

The biological safety cabinet is certified on an annual basis by an outside contractor approved of by USC EHS department.

5. Personal Protective Equipment (PPE):

Describe any PPE that must be worn when performing the procedure. This may include lab coats, gloves, and face/eye protection. Protective clothing should be removed before leaving for non-laboratory areas.

The following PPE is worn at all times in the teaching lab by students and instructor when bacteria are handled:
Standard lab coat, which is stored in individual storage bags and do not leave the lab during the semester. At the end of the semester, all lab coats are sterilized in the autoclave and may then be returned to the student for washing prior to reuse.
Nitrile gloves provided by the NSE department in 3 sizes. Proper procedures for removal of gloves and disposal are provided to the students.
Safety glasses are required during lab. Z-87 approved safety glasses with side shields are required and also do not leave lab unless properly sanitized with alcohol solutions and washed with soap and water.

6. Sample Transport:

Are BSL-2 agents transported between labs and/or buildings?

		YES
	X	NO

If YES, describe the procedures for transporting samples (i.e. durable and leak-proof secondary containers).

7. Biological Waste Disposal:

Describe how each type of biological waste generated during the procedure will be properly disposed (i.e. autoclave, sharps container, chemical disinfection). Materials to be decontaminated outside the immediate lab must be placed in a durable, leak-proof container and secured for transport.

Biological waste generated in the microbiological teaching labs shall be handled in the proper aseptic manner. All final materials shall be collected in a designated biological hazard area properly labeled as a biohazard area by orange labels with the biohazard symbol. Items may be in test tube racks, aluminum buckets and/or clear biohazard bags. All contaminated materials are autoclaved at EPA prescribed time, pressure and temperature in autoclave bags, buckets, test tube racks prior to disposal. The autoclave utilizes a 30 minutes cycle at 121^{o}C temperature. Used agar plates will be placed in stainless steel bucket to prevent overflow into the autoclave unit and to serve as a catch area. Buckets are properly label with all biohazard labels. Heat sensitive tape that turns from purple to blue during the autoclave cycle is designated with a W to indicate waste materials.

After the autoclave cycle, the liquid materials are disposed of down the sanitary drain, the plastic items are disposed of in the common trash container in clear bags and the reusable items are washed in the mechanical dishwasher prior to reuse.

On a quarterly basis, spore testing is performed on the autoclave to insure the proper pressure, temperature and time cycle is adequate for sterilization. A log of the results is available for examination.

All sharp containers are disposed of by proper boxing and ultimate disposal by Stericycle however sharps are not utilized in the microbiology labs.

8. Sharps:

Describe each type of sharps device utilized during the procedure. These may include needles, scalpels or glass pipettes.

No sharps are utilized in the microbiology lab. The Upstate campus needles, toothpicks and other possible contaminated small items are disposed of in a sharps container which does not allow for reuse or exposure. These sharp containers are collected in the Hazardous Waste location in room 102B of the Smith Building where the containers are sealed and then discarded in cardboard biohazard boxes for ultimate disposal by Stericycle.

Describe specifically how each sharps device is used, including any special precautions that may be necessary to reduce the risk of exposure. For instance, needles must not be bent, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

N/A

9. Decontamination:

Describe the decontamination of work surfaces and lab equipment. For instance, all work

surfaces will be decontaminated after completion of work and after any spill of potentially infectious material.

All lab bench surfaces (including biosafety cabinet) are disinfected before and after each session with 70% ethanol. Excess solution may be wiped up with a paper towel and disposed in the trash container.

Describe the specific type of disinfectant(s) that will be used.

A 10% Bleach Solution is utilized for all spills. The bleach solution is made fresh each week on Monday. Also available, is a 70% ethanol for routine disinfection of benches and can be utilized if bleach solution is not available.

10. Labeling/Signage:

Describe any required labeling and signage. For instance, Biohazard/BSL-2 signage on the lab entrance door and Biohazard labels on all appliance being used to store BSL-2 agents/materials.

All doors into the microbiology lab are labeled with Biohazard/BSL-2 signage as required by USC EHS department. In addition, all areas that contain microbiological specimens are labeled with the universal Biohazard label in the lab including refrigerators, incubators and designated biological areas where used specimens are to be autoclaved.

11. Biological Spill Clean-up:

Describe the procedures that will be followed in the event of a spill involving biological materials. The procedure should include the materials necessary for clean-up and the type of disinfectant that will be used. A copy of the spill clean-up procedures should be conveniently available with other biosafety resources in the lab.

Spills are decontaminated by the following steps:
The instructor cleaning up the spill must put on the appropriate personal protective equipment (minimum PPE includes gloves and lab coat). The spill is immediately covered with paper towels. A 10% bleach solution is applied to the perimeter of the spill first, and then soaked to the middle of the contained spill area. The bleach is allowed to maintain contact for 20 minutes undisturbed. After 20 minutes, the instructor utilizing appropriate PPE will wipe up the decontaminated material and place in a clear autoclave bag for proper autoclaving. Paper towels and any other contaminated materials must be autoclaved. Any spill containing broken vials and/or glassware containing bacterial cultures will also be cover with paper towels and have a 10% bleach solution added beginning with a the outside perimeter. After a contact period of 20 minutes, the instructor will utilize proper PPE and sweep the glassware and paper towel into a disposable dust pan and place the items inside a puncture-resistant biohazard sharps container. The container will be sealed and removed for proper disposal.

12. Training:

List any general training requirements for personnel performing this procedure (i.e. Laboratory Safety Training, Biosafety Training, Bloodborne Pathogens Training). Also, describe any training provided by the laboratory supervisor to ensure that lab personnel are adequately trained regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

All students enrolled in microbiology laboratory, lab assistants and instructors are trained with a Powerpoint presentation of Laboratory Safety and Biosafety. The Safety training includes the appropriate use of PPE, disinfection, procedures, decontamination procedures, transfer techniques, location and use of safety equipment (safety shower, eye wash station, first aid kit, MSDS, Biosafety Manual, emergency phone, fire extinguisher, fire blanket, etc.)

13. Vaccinations/Treatments:

List recommended vaccinations, skin tests, other medical prophylactic treatments, or medical surveillance necessary for working with this agent.

None recommended at this time.

14. Injury/Exposure Response:

Describe the response procedures that will be followed in the event of a laboratory-acquired injury/exposure. Include any initial treatment (i.e. washing exposed area), where to seek medical treatment, and any necessary emergency contact numbers.

In the event of any injury occurring in the lab (minor or major) an injury incident form will be completed and turned into the USC-Upstate Laboratory Manager- Karen Reece. In the event of skin exposure, the exposed area will be washed with water and antimicrobic soap. If the skin has been broken, the area will be washed and treated with topical antibiotic ointment and covered with a bandage. The student will be referred to Health Services for further treatment if necessary. In the event that eye exposure occurs, then the eye will be flushed with water for 15 minutes using the eyewash station. The condition will be accessed at that time. If further treatment is necessary, the student will be referred to Health Services. If the injury is critical in nature 911 will be called.

15. Laboratory Facilities:

Check each of the following that are readily available in the laboratory where the BSL-2 agent is used:

	X	Eyewash station
	X	Door(s) with locks

	X	Sink for hand washing
	X	Furniture that can be easily cleaned/decontaminated
	X	Biological safety cabinet(s) with updated annual certification

CERTIFICATION OF UNDERSTANDING:

All laboratory personnel working with the biosafety level 2 agent described in this Biosafety Registration Form will be required to sign the following.

I have fully read all sections of this Biosafety Registration Form for work involving biosafety level 2 agents. I understand all the information included in this form and agree to follow the described procedures. I understand that I may ask questions or request additional information from my laboratory PI/supervisor if I have concerns regarding the duties I will be performing, or the necessary precautions to prevent exposures. I also understand that I may contact the Department of Environmental Health and Safety to discuss any concerns with the implementation of this registration form.

Name	Signature	Date	PI Initials

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IBC Use Only		
Biosafety Officer Review Date:	**March 16, 2009**	
All revisions recommended by the Biosafety Officer have been appropriately addressed?		
	X	YES
		NO

Principal Investigator must maintain a signed copy of their registration form that indicates all revisions recommended by the BSO were appropriately addressed.