Sam Houston State University

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Institutional Biosafety Committee

Protocol Application

For all questions requiring you to check a box, please print application to complete these questions. Once the application is completed and signed, please email the completed and signed application to Sharla Miles (sharla_miles@shsu.edu)

SECTION A Principal Investigator and personnel information (please type or print)		
***P.I. Name*:** **Dr. Rachelle Smith**	***Title*: Director, Analytical Lab Ser**	***Dept*: TRIES Research Centers**
***Phone No*:** **936-294-3714**	***Alternate Phone No.*:** **936-661-1343**	***Fax*:** **936-294-3822**
Building and Lab Room No(s): **TRIES (SS3) Room 173B/C**	***E-mail:*** **env_rxs@shsu.edu**	
Emergency contact name: Rachelle Smith (936-294-3714) Autumn Smith-Herron (936-661-7766) Chad Hargrave (936-294-1538)		

Protocol Title/Course Number and Title: Efficacy of [redacted] against the plant fungus *Fusarium oxysporum* f. sp. *cubense*

★ New Protocol ☐ Teaching Protocol ☐ Renewal Protocol ☐ Amendment Protocol

Principal Investigator Acknowledgment

I accept responsibility for:

The safe use of all potentially infectious organisms at Biosafety **Level 2** and have informed all personnel of the risks of exposures while working with these organisms.

The conduct of this research is in accordance with The United States Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine Containment Facility Guidelines for Fungal Plant Pathogens.

All personnel have been informed of potential risks, and proper laboratory practices for working safely with human blood, body fluids, tissues, cell lines, and plant pathogens using Biosafety level 2 practices and procedures.

Rachelle Smith 03 Sep 2021

Principal Investigator (Signature) Date

Department Chair [Or Academic Dean if Chair is PI] (Signature) Date

For purposes of this registration, biohazardous materials are defined as any organism known to or suspected of causing infection in humans, and a toxin is a proteinaceous poison, which is highly toxic to humans. Experiments using biohazardous materials and toxins should follow the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) Guidelines (4th Edition-1999).

Experiments using recombinant DNA technology should follow the NIH Guidelines for Research Involving Recombinant DNA (rDNA) Molecules, (April 2002).

The Principal Investigator (PI) is responsible for completing the appropriate parts of this registration document. The Sam Houston State University Institutional Biosafety Committee (IBC), in conjunction with the Environmental Health, Safety and Risk Management Department (EHS & RM), maintains a registry of all laboratories and personnel working with human pathogens, and/or toxins, human blood, body fluids, and tissues, and recombinant DNA technology.

The PI is also responsible for notifying EHS & RM when work with any potentially infectious material is terminated or when other significant changes occur, such as changes in protocol, personnel, or relocation of the laboratory.

This registration document is to be forwarded to EHS & RM (EHS Specialist) prior to the initiation of work. Each individual listed should be informed of the potential hazards associated with this work, the appropriate safety practices to be used, the availability of medical programs, and applicable training requirements.

EHS & RM conducts an annual survey of registered laboratories to review practices and procedures. The survey is not intended to negate the responsibilities of the PI in supervising work with potentially infectious or hazardous materials.

RISK GROUPS AND BIOSAFETY LEVELS should be determined using the BMBL and the NIH rDNA Guidelines. Additional information for infectious agents can be found in the American Biological Safety Association (ABSA) website. **Completion of ALL sections of this application are required for ALL biosafety levels.**

SECTION B Brief description of the research understandable to scientists working in different fields.

★ New Protocol **☐ Teaching Protocol** **☐ Renewal Protocol** **☐ Amendment Protocol**

Title of protocol/ Course Number and Title: Testing the efficacy of differing concentrations of a fungicide ([redacted] formula) on fusarium oxysporum f. sp. cubense.

This project will use: **★ Biohazardous Material** **☐ Biological Toxins** **☐ Recombinant DNA**

B.1. *Provide the date when you propose to begin research and the date when you anticipate research will be completed:*

B.1.a. *Proposed start date:* 15 September 2021

B.1.b. *Anticipated completion date:* 01 October 2023

B.2. *General description of research:*

An in-vitro study testing the efficacy of differing concentrations of [redacted] formula will be conducted to evaluate them as preventative agents against banana wilt disease.

B.3. *Hypothesis:*

The [redacted] is an effective fungicide against the plant pathogen *Fusarium oxysporum* f. sp. *cubense*

B.4. *Types of biological agents and toxins, their quantity, duration of experiment, and/or the rDNA technology to be applied:*

Agar cubes containing viable cells (50 µl) of *Fusarium oxysporum* f. sp. c*ubense* will be cultivated on potato dextrose agar plates at 25 degrees C for one week. Five mm diameter agar plugs containing actively growing mycelium of the fungal isolate will be transferred to PDA amended with five different concentrations of [redacted] The culture plates will be sealed with breathable tape and kept in secondary containment when not in the BSC. Each culture plate holds approximately 20 mL of agar (nonamended and amended PDA) and each TM concentration will be run in triplicate. The duration of the experiment is anticipated for no more than four weeks. During the experiment, culture plates will be kept sealed, left in secondary containment, and stored

in the incubator until the conclusion of the experiment. The inoculated plates will be secured in a biohazard bag, decontaminated using steam heat, and disposed in the larger biohazard box.

B.5. *Significance of the project:*

Efficacy studies against exotic plant pathogens are essential to aid the USDA in mitigation efforts of plant pests that have a negative impact on agriculture activities.

B.6. *Please include any additional information that may assist the IBC in the review of this protocol (e.g., description of experimental design, procedures, etc.):*

This procedure will be performed in the BSC located in 173 B

Aliquots of 50 µL of cells will be maintained on potato dextrose agar (PDA) or PDA amended plates for a short period of time.

The [redacted] will be added to the plates containing *Fusarium oxysporum* mycelium to determine its efficacy.

Upon completion of the experiment all plates will be autoclaved and disposed of in a biosafety approved container.

SECTION C Use of recombinant DNA technology ★ **Not Applicable**

Prokaryotic Hosts/ Eukaryotic Cells *List Strains*	*Vector*	*DNA Insert*	*Relevant section of NIH Guidelines*	*Physical Containments*
N/A	**N/A**	**N/A**	**N/A**	**N/A**

If viral vector is to be used will infectious virus be generated? **N/A** ☐ No ☐ Yes

Will studies include attempts to obtain expression of a foreign gene, other than those used for selection purposes?

★ No ☐ Yes what protein _____

SECTION D Potential human pathogens and/or toxins (please provide information for each microorganism and or toxin used, use additional space if needed) ☐ **Not Applicable**

Organism: ***Fusarium oxysporum* f. sp. *cubense***	*Strain:* *strain designation 23486 (fungi)*	*Volume used:* ***50 µl***	*Risk group:* **RG1**
Biological Toxins: **N/A**		*Volume used:* ***50 µl***	*Risk group:* **RG1**

Is organism concentrated? ★ No ☐ Yes

Specify methods: ☐ Centrifugation ☐ Filtration ☐ Precipitation ☐ Other _____

Containment equipment available:

Biological Safety Cabinet: Class II A2_____ Last Certified: 03/2021

☐ Containment Centrifuge ☐ Other ___-80 C freezer for sample storage

SECTION E Use of animals

Are animals used in this project? ★ **No** ☐ **Yes** Date IACUC Approval (if applicable) _____

List all animals used in the project	*Organism, toxin, or rDNA introduced*	*Routes of administration*

Types of animal tissue handled and/or animal cell lines:

SECTION F Handling of Human Products (requires BSL-2 practices)

Are human samples used in this project? x **No** ☐ **Yes** Date IRB Approval (if Applicable) _____

Type of human samples manipulated:

☐ Cell lines Blood ☐ Tissues ☐ Urine ☐ Spinal Fluid ☐ Serum ☐ Feces ☐ Semen

☐ Other _____ Specify_____

Type of manipulations:

☐ Centrifugation ☐ Bleeding/Mixing ☐ Dissection ☐ Sonication Pipetting

Other Decantation/Blot drying (at the conclusion of each wash step – necessary for accurate results)

SECTION G Safety, Security, and Training Plan - Use the BMBL as a guide only to write your specific safety procedures. Follow the outline below for the items that are applicable to your project (please put NA in all the rest).

If you are working with animals describe in detail the safety protocol for handling infected animals in the animal care facility.

G.1. Training Plan:

All personnel involved with this project has completed online CITI training on biosafety practices. All potential additional employees will receive online CITI training on biosafety practices before the onset of work. Personnel training will also include protocols involved with sample receipt, handling, storage, and disposal of plant, pest, biological control agents, and noxious weeds organisms as well as laboratory safety. Each individual is responsible for working safely and abiding by applicable safety guidelines. All personnel shall a) become familiar and adhere to the biosafety plan and all laboratory procedures, b) comply with safety guidelines and procedures required for the task(s) performed, c) report unsafe conditions to the supervisor or Environmental Health and Safety Office, d) seek guidance when uncertain about safe handling, storing, or disposing of biohazardous material.

Dr. Autumn Smith-Herron will ensure the proper training of all personnel working on this project on the safe handling of biohazardous organisms.

All personnel will adhere to the biosafety guidelines established within the laboratory biosafety manual.

Dr. Autumn Smith-Herron will maintain a training logbook for all personnel (including annual renewal training).

Personnel working on this project include Dr. Rachelle Smith and Jessy Stone

Dr. Jeremy Bechelli will train personnel on correct procedures for working within biological safety cabinets

G.2. Security Plan:

G.2.a. Access to the laboratory:

Research will be conducted at the Sam Houston State University TRIES building located at Sam South in laboratory room 173B/C. The TRIES building remains locked during off hours and access is controlled by SHSU. Access to the laboratory, which always remains locked, is restricted to authorized personnel only. Signage on the outside of the door includes a biohazard sign indicating the agents present, the biosafety level, contact information for the PI, emergency contacts, and a list of PPE required for laboratory entry.

A sign will be placed on the door to also indicate immunocompromised individuals should not enter when the organism *Fusarium oxysporum* f. sp. *cubense* is in use.

The laboratory is already certified for work with BSL-2 pathogens.

G.2.b. Access to the biological agents:

A plant pathogen BSL-2 agent, *Fusarium oxysporum* f. sp. *cubense* (FOC), will be used and contained within the assigned laboratory where testing will be conducted. FOC stock and experimental samples will be stored in a -80 C freezer until use. The freezer is also labeled with a biohazard symbol and is locked to restrict access. Only authorized trained personnel will have access to the agent.

G.3. Safety Plan:

G.3.a. Specific Laboratory Practices:

The ampule of FOC purchased from ATCC will be received by the PI and stored in the ThermoScientific TXS freezer located just outside laboratory room 173 (B/C) at -80 °C for no more than one week. When ready to begin the experiment the necessary materials (forceps, hotplate, beaker(s), and prepared potato dextrose agar (PDA) plate) will be gathered and placed on the work bench outside the BSC. The BSC will be turned on and allowed to run for 4 minutes to purge the cabinet of particulates. The BSC will be disinfected with a 10% bleach solution followed by a water rinse. An adsorbent plastic-backed pad will be laid down to protect the worksurface and facilitate cleanup. The frozen ampule will be removed from -80 °C storage, wiped with disinfectant, and placed in a secondary container with a secure lid (e.g., Tupperware ® dish) that has been disinfected with the 10% bleach solution and filled with absorbent material (e.g., paper towels) and taken to the BSC located in laboratory room 173B. The external surfaces of any equipment and supplies will be wiped down with 10% bleach disinfectant and placed inside the BSC. Inside the BSC, the ampule will then be removed from secondary containment and thawed in a water bath maintained at 25 to 30 °C for approximately 5 minutes. Immediately following thawing, the ampule will be wiped with 70% ethanol and 2-3 agar cubes (50 µL) of the *Fusarium oxysporum* f. sp. *cubense* will be aseptically transferred onto the PDA plate. The inoculated plate will then be sealed with breathable tape (e.g., Breathe-Easy ® sealing membrane) around the outside to prevent exposure to moisture or drips that may seep out as well as keeping the lid securely attached to the base. The ampule, if not empty, will be wiped with disinfectant (10% bleach), placed in secondary containment, and transferred back into the -80 °C freezer. If the ampule is empty it will be decontaminated in a pan containing 10% bleach solution for a minimum of 10 minutes, placed in a biohazard bag, autoclaved at 121 °C for a minimum of 30 minutes and disposed of in the larger biohazard bag/box located in the laboratory. The sealed, inoculated PDA plate will be wiped down with disinfectant (10% bleach), placed in the secondary container (upside down) with the lid securely attached and again wiped down with disinfectant. The container will be transported to the incubator located in laboratory room 173C and both sealed container and plate set inside the incubator. The sealed container and plate will be incubated at 25 °C for one week. After the one week, the container containing the FOC inoculated plate in the incubator will be wiped down with disinfectant (10% bleach), removed from the incubator and transported to the BSC, which will be disinfected as outlined previously. PDA amended and non-amended (control) prepared plates will be arranged in the BSC and using a plastic straw without an applicator stick, agar cores will be cut and removed from the amended and non-amended plates using the following procedure. Inside the BSC the FOC inoculated plate will be removed from secondary containment and wiped down with disinfectant prior to opening. The tape, with minimal movement, will be

cautiously and carefully removed from the FOC inoculated plate to decrease potential release of FOC spores, the tape will then be disposed of in a biohazard waste container in the BSC. Using plastic straws with a wood applicator sticks placed inside the straw, five mm diameter agar plugs are removed from the FOC inoculated plate as follows: a) the middle portion of the straw is grasped and squeezed to hold the stick within the straw, b) the applicator stick is pulled up into the tube approximately 1-2 cm from the bottom of the straw, c) the straw is inserted and punched down into the actively growing mycelium of the FOC inoculated plate, d) the straw is rolled between the fingers to cut the core and then tilted to break the adhesive force of the agar plug, e) the straw is lifted out and the tip containing the FOC agar plug is placed in to the hole in the recipient plate, f) the applicator tip is gently pushed down against the plug while the straw is slowly lifted out of the agar hole (Fusaro, 1972). Agar plugs are placed in the labeled recipient plates in a completely randomized design. All plates, including the FOC inoculated one, are sealed with breathable tape. All plates will be wiped down with disinfectant (10% bleach). The FOC inoculated plate will be secured in a biohazard bag, placed in a secondary container, wiped down with disinfectant, taken from the BSC, placed in the autoclave, and decontaminated with steam heat. The biohazard bag will be removed from the autoclave and placed in the larger biohazard bag/box located in the laboratory. The PDA amended and control plates are also placed in a secure secondary container, transported to the incubator, and set in the incubator (again, container and plates). Plastic straws and applicators will be placed in a pan containing 10% bleach (in the BSC) and allowed to sit for a minimum of 10 minutes. These items then will be placed in a biohazard bag and decontaminated further using steam heat. The bag will be removed from the autoclave and placed in the biohazard box located in the laboratory. Samples are incubated at 25 °C and inspected daily for growth for up to 4 weeks. To inspect for growth, the secondary container inside the incubator is wiped down with disinfectant (10% bleach), moved to the BSC, the container is opened, and plates are removed carefully and inspected. If growth is detected, the radial mycelial growth will be measured in two perpendicular directions. Please note that tape will not be removed from the plates nor plates opened at any time during the remainder of the experiment to reduce the likelihood of release of spores. Once the daily inspection is complete, plates are wiped down with disinfectant, placed back into the secondary container and returned to the incubator. Upon completion of the experiment the plates will be placed in the secondary container, wiped down with disinfectant (10% bleach), secured in a biohazard bag, and decontaminated with steam heat in the autoclave. The decontaminated bag will then be placed in the biohazard box located in Rm173B. The BSC will be disinfected with 10% bleach prior to each use and at the completion of each use.

In the event of a building evacuation, the blower motor of the BSC will not be turned off. All containers will be closed and left in the BSC. If time permits the materials will be wiped down, put in secondary containment, and returned to the incubator. All PPE will be removed while still in the laboratory and hands are washed before leaving the laboratory area. Once given permission to return into the building, work will be resumed.

G.3.b. Personal Protective Equipment Required:

Laboratory personnel will practice standard precautions to include washing hands, wearing disposable lab coats, gloves, eye protection, long pants, and closed toe shoes. While working inside the laboratory and BSC, gloves, disposable lab coats, and eye protection are required. Face shields and disposable surgical masks are available and may be worn by personnel for the duration of the experiment. All experiments will be performed inside the Class II BSC using aseptic technique. Contaminated gloves or PPE will be removed and secured in a biohazard bag then decontaminated using steam heat before placing in the larger biohazard

bag/box located in the laboratory. Non-contaminated PPE will be removed and placed in large biohazard bag/box before leaving laboratory area. Hands will be washed before leaving the laboratory.

G.3.c. Containment and Safety Equipment Used:

All experiments with *Fusarium oxysporum* f. sp. *cubense* will be performed inside of a Class II BSC using aseptic technique. *Fusarium oxysporum* f. sp. *cubense* will be stored in its original vial and kept at -80 °C until used but not to exceed one week. The inoculated plates (PDA amended or non-amended) will be secured with breathable tape and kept in secondary containment when not in the BSC. To avoid using sharps, the agar plugs will be handled using plastic straws and wood applicator sticks as outlined in Fusaro, 1972. Used straws and sticks will be placed in a pan containing 10% bleach solution, secured in a biohazard bag, decontaminated using steam heat, then disposed of in the larger biohazard bag/box. Secondary containers will be rigid and are secured with a lid. Biohazard bags and a large biohazard bag/box are available and located in laboratory 173B. Disinfectant used will be a 10% bleach solution and will be used on all surfaces prior to and at the conclusion of each task performed for this experiment. Decontamination using steam heat will be performed using an Easyclave autoclave that is properly maintained and calibrated annually.

Full face shields and surgical masks are available for personnel to use if desired, however, it is not anticipated that any aerosols will be generated, and the only liquids being used in this experiment are the potato dextrose agar dissolved in water (to prepare the agar plates) and the [redacted]

G.3.d. Decontamination and Spill Clean-up Procedures:

Decontamination of used plates and any consumables (e.g., plastic straws, wood applicator sticks, gloves, towels, etc.) will be performed by securing the items in a biohazard bag, autoclaving the bag at 121 °C for a minimum of 30 minutes, and placing the decontaminated bag into the larger biohazard bag/box located in laboratory 173B. All laboratory working surfaces, including the BSC will be cleaned with a 10% bleach solution every day (before and after each use). Spills within the laboratory, outside the BSC, or other physical containment devices will be reported to the supervisor or Principal Investigator and documented. Reported and documented spills are any spills that may contain the presence of potentially infectious materials or the possibility of splashes and generation of aerosols or airborne particles.

Spills within the BSC require that the blower motor be left on. The spill is covered with absorbent towels and disinfectant (10% bleach) is copiously applied in a circular motion. The spill is wiped up as much as possible with the aid of tongs. The absorbent material is then secured in a biohazard bag. More absorbent towels will be laid down and more disinfectant is applied to the towels and allowed to set for 10 minutes. Any bleach residue left in the BSC is rinsed with water and all cleanup materials secured in the biohazard bag. Any objects within the BSC that come in contact with the spill will also be decontaminated by wiping down the exterior surfaces with disinfectant. Spills that are large or difficult to clean such as those in the drain spillage trough, require the assistance of the Safety Office and they will be contacted.

In the event of a spill outside the BSC and within the laboratory, all work will immediately stop, and a sign will be posted on the doors notifying all personnel of a spill. The room will be evacuated for 30 minutes to allow droplets to settle. After 30 minutes the PI will don PPE (gown, gloves (double glove) shoe covers, and a surgical mask to enter the room). The spill will become contained with absorbent towels or pads and a 10% chlorine bleach solution, or any appropriate disinfectant around the perimeter of the spill working toward the center of the

spill to inactivate infectious materials. The disinfectant will be allowed to stand for 30 minutes before removing the absorbent material and placed in a red biohazard bag. Glass or other sharps will be removed with tongs and discarded in an approved sharps container. Once all materials have been removed, the space will be decontaminated again with an appropriate disinfectant. If a mop is used, it will be soaked in fresh disinfectant for 30 minutes before rinsing for reuse. Finally, PPE will be removed, placed in the biohazard bag, and wash hands thoroughly with soap and water. The biohazard bag(s) will be decontaminated using steam heat and discarded in the larger biohazard bag/box.

A portable, multi-use biohazard spill kit containing PPE, absorbent pads, germicidal wipes, and other supplies for cleanup is available.

G.3.e. Transfer and/or Transport of Biohazards Between/Outside the Laboratories:

The frozen ampule will be removed from the -80 degree C storage and placed in disinfected secondary containment (covered Tupperware dish) in order to transport to the BSC. Ampule will only be removed from secondary containment when located in the BSC. The ampule, if not empty, will be placed in secondary containment and transferred back into the -80 degree C freezer, If the ampule is empty, it will be decontaminated in a 10% bleach solution, autoclaved, and disposed of in the biohazard bag/box located in the laboratory. Any materials that need to be transferred between certified labs will be maintained in leak proof secondary containment with a sealed lid or closure containing absorbent material (examples are Tupperware plastic containers that can be fully cleaned in the event of a spill). Containers will be disinfected between uses using 10% bleach solution and the outside of the containers will be disinfected before removal from the BSL-2 laboratory.

G.3.f. Handling of Hazardous Waste:

Inoculated plates will be sealed with breathable tape, wiped with disinfectant (10% bleach) prior to handling, and kept in disinfected, secure secondary containment (except when in the BSC), including while in the incubator, to eliminate the chance of any escape of the FOC spores. Contaminated plates, materials, gloves, absorbent materials, etc., will be secured in biohazard bags and decontaminated using steam heat as outlined previously. Autoclaved biohazard bags are then placed in the larger biohazard bag/box located in the laboratory along with any noncontaminated PPE. When this biohazard box is 2/3 full, the bag is tied to secure contents and the box closed. Stericycle, the outside disposal vendor for biohazardous waste, picks up the boxes during the quarterly cycle. Anything being removed from the BSC will be wiped down with disinfectant before taking out of the BSC including biohazard bags designated for steam heat decontamination. Spores from FOC have been shown to be inactivated by bleach disinfection and steam heat decontamination.

G.3.g. Medical Surveillance:

There is no need for specific medical surveillance other than to understand the signs and symptoms of a potential exposure. Personnel will be made aware of signs and symptoms of exposure which include signs of keratitis (eye redness, eye pain, excessive tears, or discharge from eyes), onychomycosis (nail discoloration, thickening or distortion of nails, pain and discomfort, foul smell), dermatitis (itchiness, rash or swollen skin, lesions, blisters), and allergies (sneezing, itchy eyes, wheezing, shortness of breath, swelling of lips, tongue, eyes, or face). Onychomycosis may be treated with itraconazole, terbinafine, ciclopirox olamine lacquer, or topical antifungal agents. *Fusarium* species infections occur superficially, locally invasive, and disseminated. In immunocompetent individuals, keratitis and onychomycosis

are the most common infections. Immunocompromised individuals are at increased risk of fusariosis and is typically invasive and disseminated (Nucci, et. al., 2020). Infections due to FOC are predominantly found to be superficial and subcutaneous occurring on the skin, eyes, and nails (Al-Hatmi, et. Al, 2016).

The PI will instruct personnel to notify their physician that they were exposed to *Fusarium oxysporum* f. sp. *cubense* if they need/seek medical attention.

H. Special Safety Practices in the Animal Care Facility:

N/A. We will not use the animal care facility

References:

Fusaro, R.M. 1972, "Inoculation Technique for Fungus Cultures", Applied Microbiology, Vol 23, No. 1, p. 174-176.

Nguyen, T.V., Tran-Nguyen, L.T.T., Wright, C.L., Trevorrow, P., and Grice, K. 2019, "Evaluation of the Efficacy of Commercial Disinfectants against *Fusarium oxysporum* f. sp. *cubense* Race 1 and Tropical Race 4 Propagules", Plant disease, Vol 103, p. 721-728.

Gupta, A.K., Baran, R. and Summerbell, R.C., 2000, Apr, "Fusarium infections of the skin", Current opinion in infectious diseases, Vol 13, No. 2, p121-128.

Al-Hatmi, A.MS, Hagen, F., Menken, S. BJ, Meis. J.F., de Hoog, G.S. 2016, "Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015", Emerging microbes & infections, Vol. 5, e124, p.1-11

Nucci, M., Anaissie, E., 2020 Dec 15, "Mycology, pathogenesis, and epidemiology of Fusarium infection", https://www.uptodate.com/contents/mycology-pathogenesis-and-epidemiology-of-fusarium-infection

Will ship or transport biohazardous material	★ No	☐ Yes
Will generate biohazardous waste	☐ No	★ Yes

The following websites contain information that can help you complete the Registration for Recombinant DNA Research Form

http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl3toc.htm CDC - Biosafety in Microbiological and Biomedical Laboratories (BMBL)
http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html NIH guidelines for work with recombinant DNA molecules
http://www.absa.org/resriskgroup.html ABSA - American Biological Safety Association Risk Group classification tables
http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf WHO 2004 Laboratory Biosafety Manual