THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that requi Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must ~~also~~ be updated at least every 3 years or when there are changes to the biohazards being use

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contac the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification fo See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR Dr. Larry Allen
→ SIGNATURE
DEPARTMENT Ophthalmology
ADDRESS PRB 504 SJHC
PHONE NUMBER 646-6000 x66366
EMERGENCY PHONE NUMBER(S) (519) 933 8018 - T. Chan fy
EMAIL larry.allen@sjhc.london.on.ca

Location of experimental work to be carried out: Building(s) South Street Hospital Annex Room(s) 117

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer fo the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: Department of Ophthalmology, UWO
GRANT TITLE(S): Treatment of bacterial corneal ulcer in a rabbit eye using intravitreal injection of moxifloxacin

✓ **PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.**

Names of all personnel working under Principal Investigators supervision in this location:

Dr. Ragkaya Mather
Dr. John Chan
Trisha Kirkpatrick

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ⊘YES O NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ⊘NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:
The only possible risk is spilling of 5 mL of bacterial inoculate to clean up as per UWO & CFIA protocol. Sterilize surface with 1% sodium hypochlorite.
(confirmed with LHSC VH Microbiology)
Please attach the CFIA permit.
Please describe any CFIA permit conditions:
_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Pseudomonas aeruginosa	⊘Yes O No	⊘Yes O No	O Yes ⊘No	0.002 L	LHSC Microbiology	O1 ⊘2 O3
	O Yes O No	O Yes O No	O Yes ⊘No			O1 O2 O3
	O Yes O No	O Yes ⊘No	O Yes O No			O1 ⊘2 O3
	O Yes ⊘No	O Yes O No	O Yes O No			O1 O2 O3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O YES ⊘NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ⊘NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Section 1.0 and 2.0? O YES ⊘NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⊘NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ⊘YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used New Zealand White rabbits (NZW)

6.3 AUS protocol # 2009-035

6.4 Will any of the agents listed be used in live animals ⊘YES, specify: P. aeruginosa O NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES Ø NO
- Pound source cats O YES Ø NO
- Cattle, sheep or goats O YES Ø NO
- Non-human primates O YES, please specify species _____ Ø NO
- Wild caught animals O YES, please specify species & colony # _____ Ø NO
- Birds O YES Ø NO
- Others (wild or domestic) O YES, please specify _____ Ø NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pc

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ~~O NO~~
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 10.0 ~~O~~ NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to atter the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 Ø2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
Ø YES, permit # if on-campus _____ (no answer from SSH)
O NO, please certify
O NOT REQUIRED for Level 1 containment

Please contact Gail Ryder x75109 (no answer)

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines an Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Haza Communication Form, found at http://www.wph.uwo.ca/

→ SIGNATURE _____ Date: April 22/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

INTRASTROMAL VIGAMOX VERSUS TOPICAL VIGAMOX IN A NEW ZEALAND WHITE RABBIT KERATITIS MODEL

Resident: Toby Chan, PGY3
Supervisors: Dr. Larry Allen, Dr. Rookaya Mather

Purpose: To compare bacterial killing of Vigamox administered as a single intrastromal corneal injection to topically administered Vigamox in the treatment of *Pseudomonas aeruginosa* bacterial keratitis.

Methods:

1) Each eye of 24 New Zealand White rabbits, will be intrastromally inoculated with 25 microlitres of broth containing ~1000 colony-forming units of Pseudomonas bacterial isolates.
2) 16 hours later, slit lamp pictures will be taken. 6 rabbits will be euthanized to determine the number of corneal bacterial colonies at the onset of therapy.
3) The remaining 18 rabbits will be divided into 3 groups:
    a. Stromal group: 6 rabbits will receive 0.05 cc of moxifloxacin injected via a 30-gauge needle into the cornea at 4 locations surrounding the ulcer. Slit lamp pictures will be taken.
    b. Topical group: 6 rabbits will receive topical moxifloxacin(0.5%) drops in both eyes every 15 minutes for 5 doses and then every 30 minutes for 14 doses.
    c. Control group: 6 rabbits will receive topical saline drops in both eyes every 15 minutes for 5 doses and then every 30 minutes for 14 doses.
    d. 1 hour following the final treatment, all animals will be euthanized and corneas will be harvested. Slit lamp pictures will be obtained. Bacterial colony counts will be determined using standard methods. Colony counts from each group will be compared.

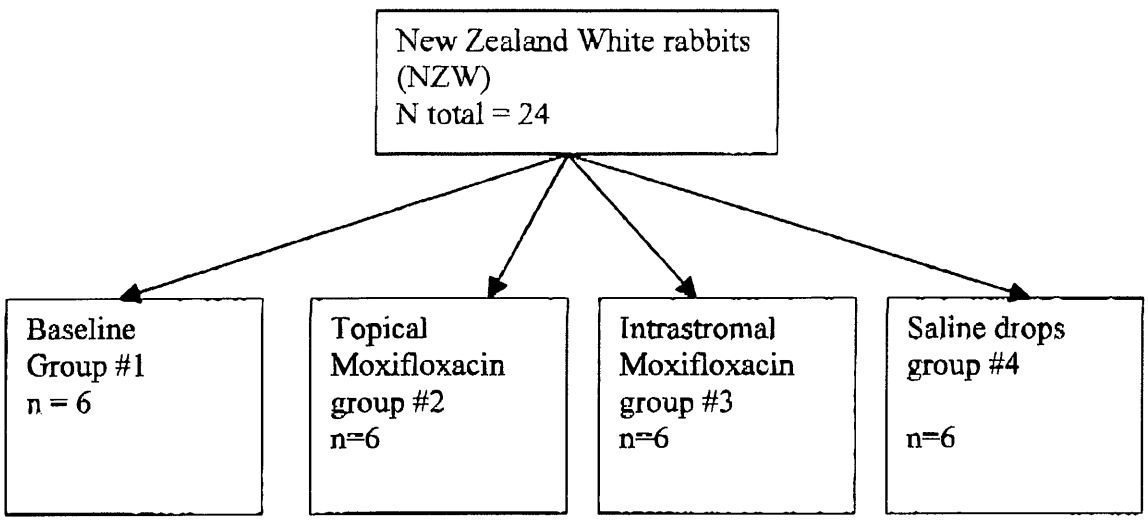
References:

Mah FS et al. **Zymar (Gatifloxaxin 0.3%) shows excellent gram-negative activity against serratia marcescens and pseudomonas aeruginosa in a New Zealand White Rabbit Keratitis Model.** Cornea. June 2007; 26(5):585-588.

Garcia-Velenzuela E et al. **Intracorneal injection of amphotericin B for recurrent fungal keratitis and endophthalmitis. Arch Ophthalmol.** Dec 2005; 123:1721-1723.

Title: Treatment of bacterial corneal ulcer in a rabbit eye using intrastromal injection of moxifloxacin

Experimental group Flow Chart
(Section D5.1)



(provided by UHJC Microbiology - Victoria Hospital)

Pseudomonas spp. (excluding B. mallei, B. pseudomallei) - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Pseudomonas* spp. (excluding *B. mallei, B. pseudomallei*)

SYNONYM OR CROSS REFERENCE: *P. aeruginosa, P. cepacia*

CHARACTERISTICS: Family Pseudomonadaceae, gram negative bacillus, aerobic, non-spore forming, some pigmented (pyocyanin, fluorescein), motile by polar flagella, variety of toxins produced

SECTION II - HEALTH HAZARD

PATHOGENICITY: 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

EPIDEMIOLOGY: Worldwide; increasing in frequency in recent years; commonly a nosocomial infection associated with contaminated instruments; 16% of nosocomial pneumonia, 12% of hospital acquired urinary-tract infections; rarely causes community acquired infections in immunocompetent patients

HOST RANGE: Humans, animals, plants

INFECTIOUS DOSE: Not known

MODE OF TRANSMISSION: Direct contact with contaminated water, aerosols or aspirations, by contact of mucous membranes with discharges from infected conjunctivae or upper respiratory tract of infected persons through contaminated objects (improperly sterilized medical equipment, contaminated IV fluids) or fingers;

INCUBATION PERIOD: Variable depending on infection; eye infection - 24 to 72 hours

COMMUNICABILITY: Can be transmitted during course of active infection

SECTION III - DISSEMINATION

RESERVOIR: Saprophyte - soil, water, decomposing matter; infected animals and humans; infected solutions - I.V., soaps, eye drops, humidifiers; organism thrives in moist conditions

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to extended spectrum penicillins, aminoglycosides, cephalosporins, fluroquinolones, polymixins and monobactams; aminoglycoside with a beta-lactam penicillin is the first line of treatment

DRUG RESISTANCE: Multidrug resistant strains are on the rise

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde; few reports of this bacteria growing in disinfectant solutions; alcohol-containing disinfectants recommended for resistant strains

PHYSICAL INACTIVATION: Inactivated by moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour)

SURVIVAL OUTSIDE HOST: Survives for several months in water with minimal nutrients

SECTION V - MEDICAL

SURVEILLANCE: Bacteriological identification of infection

FIRST AID/TREATMENT: Antibiotic therapy - aggressive treatment is necessary to avoid chronic infections; drainage of wounds; local application of antibiotic ointment or drops

IMMUNIZATION: None

PROPHYLAXIS: Antibiotic prophylaxis, not usually administered

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: No reported infections to date

SOURCES/SPECIMENS: Clinical specimens - respiratory secretions, wound exudates, blood, urine; environmental specimens - water, infected solutions (IV, disinfectants, soap)

PRIMARY HAZARDS: Accidental parenteral inoculation; direct contact of mucous membranes with infected materials; inhalation of infectious aerosols and ingestion also present a hazard

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving suspected or known infectious specimens and cultures

PROTECTIVE CLOTHING: Laboratory coat, gloves when direct contact with infectious materials is unavoidable

OTHER PRECAUTIONS: Good personal hygiene, frequent hand washing and the avoidance of rubbing eyes as a precautionary measure against eye infections

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time before clean up and disposal (30 min)

DISPOSAL: Decontaminate before disposal - steam sterilization, chemical disinfection, incineration

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: March, 2001

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-05-14