

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
**Approved Biohazards Subcommittee: October 14, 2010**  
**Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

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, 1<sup>st</sup> 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 Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto: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 form. See website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR	<u>Lars Konermann</u>
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EMERGENCY PHONE NUMBER(S)	<u>519-473-1906 (home)</u>
EMAIL	<u>konerman@uwo.ca</u>

Location of experimental work to be carried out: Building(s) B&G Room(s) 2016

\*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for 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 15.0, Approvals).

FUNDING AGENCY/AGENCIES: PrioNet  
Canada  
 GRANT TITLE(S): Structural Proteomics of Prion Aggregates

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Lars Konermann</u>	<u>konerman@uwo.ca</u>	<u>July 11, 2011</u>

The proposed experiments will require a Level 1 biosafety environment.

Our work will not involve any live organisms. Instead, we will study a special form of protein.

The following information is provided by David Wishart's laboratory at the University of Alberta, who will supply the samples for our work at UWO.

The prion material for the current project is recombinant Syrian hamster prion, called sHPrP(90-231). The prion was genetically-engineered to resemble part of a protein produced by hamsters with an added poly-His-tag tail. The gene was then transformed into *E. coli*, where it is transcribed to produce a protein fragment. The protein is obtained by lysing cells of *E. coli* and then purifying the proteins. Since the protein used in the lab is only a fragment of the protein found in nature and since it lacks many physiological components, including lipid anchors, this prion is not believed to convert into any infectious (scrapie) form. Syrian hamster protein is not known to cross the species barrier easily and the prions that we are working with are not inherently infectious. In fact, there are no documented laboratory-acquired prion infections from working with scrapie materials of any kind.

At UWO, samples will be stored in our -80 deg freezer.

Any leftover samples will be destroyed using the autoclave available in the UWO Biochemistry Department.



See E-mail

## PROJECT SUMMARY:

The central element in the development of the prion diseases is the conversion of the cellular (PrPC) into a pathological fibril-forming isoform (PrPSc). This transition is characterized by formation of the beta-sheet rich insoluble form of the protein which leads to the accumulation of amyloid fibrils in the central nervous system and eventually to death. The exact molecular mechanisms which lead to the conformational change, as well as the final structure of the fibrils, are still unknown.

We propose to characterize the prion protein conformational change and the three-dimensional structure of the prion fibrils using a structural proteomics approach: a combination of modern protein chemistry and mass spectrometry. We will apply a combination of limited proteolysis, surface modification, chemical crosslinking, and deuterium exchange with modern mass spectrometric analytical methods. The complementary information delivered by these approaches will provide a solid foundation for solving the topology of this protein complex and for determining the details of the PrPC to PrPSc transition.

Prion aggregates form due to intramolecular binding. Using state-of-the-art structural proteomics techniques to determine the 3-dimensional structure of prions, and how this 3-dimensional structure changes upon aggregation, will enable us to determine what these interacting regions are. Determining the sequence of amino acids that interact with a second prion molecule, as well as the shape of this interacting surface, will allow the development of inhibitors designed to block this interaction, thus preventing aggregation. Specific crosslinked peptides which can only form from PrPSc can be used as the basis of a screening or diagnostic test for the presence of the pathological form of this protein in humans or animals.

We have recently developed an array of novel structural proteomics methods which will allow us to overcome bottlenecks that have plagued similar challenging applications. We expect to accumulate sufficient data to serve as structural constraints in molecular modeling. This will allow us to develop a confident and unequivocal model of the prion aggregate. Each method separately cannot provide complete structural information on its own, but we believe that a *combination* of these multiple approaches will provide sufficient complementary information for solving the topology of these complexes and determining the details of the interfaces between the prion subunits. Application of these multiple innovative structural proteomics approaches ensures a high probability of success for this proposed study.

## 1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA?  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:

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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	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
recombinant Syrian hamster prion, sHPrP (90-231)	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	N/A		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

\*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?  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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		

Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input type="radio"/> No			
Rodent	<input type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No			

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

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

### 3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials?  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 Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

### 4.0 Genetically Modified Organisms and Cell lines

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

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

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

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

\*\* Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below  NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective?  YES  NO

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

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

## 5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent?  YES  NO  
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)  
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: \_\_\_\_\_  
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host?  YES  NO

5.3 How will the biological agent 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?  YES, number: \_\_\_\_\_  NO  PENDING

## 6.0 Animal Experiments

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

6.2 Name of animal species to be used \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: \_\_\_\_\_  NO

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify:

\_\_\_\_\_  
\_\_\_\_\_

## 7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)?  YES  No If no, please proceed to section 8.0

7.2 Will live animals be used?  YES  No

7.3 If yes, please specify the animal(s) used:

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

7.4 If no live animals are used, please specify the source of the specimens:  
\_\_\_\_\_

## 8.0 Biological Toxins

8.1 Will toxins of biological origin be used?  YES  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<sub>50</sub> (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\*? \_\_\_\_\_

8.6 Will any biological toxins be used in live animals?  YES, Please provide details: \_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

## 9.0 Insects

9.1 Do you use insects?  YES  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 life stage of the insect? \_\_\_\_\_

9.5 What is your intention?  Initiate and maintain colony, give location: \_\_\_\_\_  
 "One-time" use, give location: \_\_\_\_\_

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

9.7 Do you use insects that require a permit from the CFIA permit?  YES  NO  
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 10.0 Plants

10.1 Do you use plants?  YES  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?  Grow and maintain a crop  "One-time" use

10.6 Do you do any modifications to the plant?  YES  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:

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10.8 Is the CFIA permit attached?  YES  NO  
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 11.0 Import Requirements

11.1 Will any of the above agents be imported?  YES, please give country of origin \_\_\_\_\_  NO  
If no, please proceed to Section 12.0

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

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

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

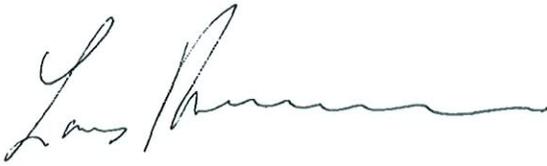
### 12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend 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 of the biological agents in Sections 1.0 to 9.0 have been trained. [The PI (Lars Konermann) has just signed up for the next Biosafety training session (July 11, 2011). All future students and PDFs involved in the project will receive training before commencing work on this project.]

SIGNATURE



**13.0 Containment Levels**

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.  1  2  2+  3

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, date of most recent biosafety inspection: \_\_\_\_\_  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): \_\_\_\_\_

**14.0 Procedures to be Followed**

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

N/A  
\_\_\_\_\_  
\_\_\_\_\_

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

accidental splash: the area will be cleaned with bleach  
\_\_\_\_\_  
\_\_\_\_\_

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and 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 Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE



Date: 24 June 2011

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

2) Safety Officer for the University of Western Ontario

SIGNATURE: \_\_\_\_\_

Date: \_\_\_\_\_

3) Safety Officer for Institution where experiments will take place (if not UWO):

SIGNATURE: \_\_\_\_\_

Date: \_\_\_\_\_

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:



**Office of Environmental Health & Safety**  
Biosafety Division

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November 27, 2006

**RE: Biosafety Precautions with Non-Infectious Prion and Prion Peptide Model Systems**

There have been several inquiries over the last couple of months from University researchers wanting to know if prion model systems using non-infectious prions or prion peptides incapable of self-amplification were exempt from the CFIA standards. In a follow up inquiry to the CFIA, the federal regulator stated that these non-infectious model systems were indeed exempt from the published containment standards but it would still be prudent to follow the guidelines listed below when working with these model systems "in the event that an intact infectious prion protein is inadvertently introduced to the laboratory through whatever means".

- Personnel should wear the following protective equipment when handling the recombinant prion: floor length pants, close toed shoes, latex gloves, buttoned-up laboratory coat or fully-tied gown, safety glasses. Safety glasses should be worn even if the person wears prescription glasses to insure full coverage (especially on the sides).
- Personnel who have had surgical or cosmetic procedures (including, but not limited to tattoos and piercings) or physical injuries (i.e., cuts, abrasions, burns, etc.) involving significant alteration to the normal integrity of the skin should not enter the research laboratory until healed. This is especially important where the area involved is on the face, head, neck, hands or arms.
- Personnel should report all potential exposures either through spills, splashes or sharp incidents no matter how slight they appear.
- Where possible, to minimize sharp hazards non-glass equipment alternatives should be employed.
- If possible, any centrifugations of the recombinant prion should be conducted using rotor safety cups which should be loaded and unloaded in a biosafety cabinet.
- Use 10% bleach as the general laboratory disinfectant. Try to leave in place for at least two minutes before wiping up. Because bleach is fairly corrosive at this concentration, immediately rinse any steel objects with water or 70% ethanol after bleach treatment.
- In the event of a spill involving the recombinant prion, the affected surface and any equipment should be treated with 10% bleach for one hour then rinsed with water. To treat a surface with bleach for one hour, have a set of old bath towels on hand to soak with the disinfectant and place on the spill site. The spill must be reported to the Environmental Health & Safety Biosafety Division.
- Potentially contaminated solid waste should be autoclaved at 134 degrees Celsius for one hour. Alternately, the solids may be treated for one hour in 2M NaOH then transferred into a container of water before autoclaving at 121 degrees Celsius for one hour.
- Potentially contaminated liquid waste should be autoclaved at 134 degrees Celsius for one hour or mixed with bleach to a final concentration of 5% bleach for one hour.

If you have any questions or concerns in regards to these guidelines, please contact the undersigned.

Daniel C. Dragon, Ph.D.  
Biosafety Officer

----- Original Message -----

**Subject:**Re: BARF - autoclave use (Koneremann lab)

**Date:**Mon, 27 Jun 2011 15:34:45 -0400

**From:**Lars Koneremann <konerman@uwo.ca>

**To:**Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer:

I just had a quick chat with Kyle - he thinks that it is likely no problem to run at the higher temperature.

But I think we'll be fine, even at the lower temperature / or even solely based on chemical treatment. Citing from the Alberta document I sent you earlier:

Potentially contaminated solid waste should be autoclaved at 134 degrees Celsius for one hour.

\*Alternately\*, the solids may be treated for one hour in 2M NaOH then transferred into a container of water before autoclaving at 121 degrees Celsius for one hour.

Potentially contaminated liquid waste should be autoclaved at 134 degrees Celsius for one hour \*or\* mixed with bleach to a final concentration of 5% bleach for one hour.

In response to your questions:

1. How frequently will you autoclave waste?  
Not very often - maybe once a month

2. What is the volume of waste you need to do (this will determine the size of autoclave, for example).  
Minimal volumes ... I'm guessing less than 1 L per month.

Please let me know if you have any additional questions.

Thanks,

Lars



E-mail