

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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 (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, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact 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 form. See website: www.uwo.ca/humanresources/biosafety/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	Dr. Ewa Cairns
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Location of experimental work to be carried out :

Building : SDRI	Room(s): 220, 221
Building : _____	Room(s): _____
Building : _____	Room(s): _____

***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: Arthritis Society

GRANT TITLE(S): Arthritogenicity of the immune response to Citrullinated Antigens

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Sheri Saunders	ssaunde5@uwo.ca	Sept 18 2007
Dr. Maud Racape	mracape@uwo.ca	Oct 24 2011
Radha Joseph	rjoseph5@uwo.ca	Oct 3 2010

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

Arthritis Society Grant Summary

Objective: To Determine the mechanisms underlying the pathogenesis of Rheumatoid Arthritis

Background: Rheumatoid Arthritis (RA) is a common, chronic autoimmune disease characterized by inflammation, cartilage degradation and bone injury in the joint. The cause of RA is unknown. The disease in humans is strongly linked genetically to the expression of MHC class II molecules containing the shared epitope (SE) a consensus amino acid sequence in the peptide binding site of antigen presenting cells. It is also linked specifically to the expression of antibodies to post-translationally modified citrullinated peptide/protein antigens containing citrulline (Cit) instead of arginine. Among these are citrullinated fibrinogen and vimentin found in RA synovium. We demonstrated that citrullinated human fibrinogen (CithFib) when injected to SE (DR4) tg mice, causes cit-specific T and B cell activation and arthritis. While the frequency of CithFib-induced arthritis in DR4 tg mice is about 40% both arthritic and non-arthritic mice generate similar Cit-specific immune responses. This finding in DR4 tg arthritic mice is relevant because it parallels pre-clinical RA in humans who have Cit peptide (CCP2)-specific antibodies and remain healthy for several years before the onset of the disease. This finding raises important questions of whether, how and when Cit-specific immune responses are pathogenic. We began to address this question in CithrFib immunized arthritic vs. non-arthritic DR4 tg mice. We now have preliminary direct evidence that both immune cells sensitized to Cit and serum from DR4 tg arthritic mice but not non-arthritic mice cause arthritis following transfer to naïve syngeneic recipients. This arthritogenicity is dependent on the intra-articular injection of CithFib which is not itself arthritogenic. Furthermore, purified serum Cit peptide reactive IgG antibodies from RA patients but not normal human IgG cause arthritis in FcγRIIb deficient mice.

Hypothesis: The development of RA requires two hits: 1) a systemic immune response initiated through Cit peptide/protein antigen release by extra-articular apoptosis in genetically susceptible individuals with SE; 2) the later intra-articular apoptotic release of Cit antigens triggers an articular immunoinflammatory response in Cit-sensitized individuals with the SE.

Research Plan: We propose to continue testing our hypothesis on how Cit-specific immune responses cause arthritis, by exploring the nature of the synovial Cit antigen targets of this response and the critical immune effector mechanisms responsible for the induction of arthritis in arthritis-responder vs non-responder DR4 tg mice. We will also examine in more detail how Cit-specific IgG antibody from human RA patients causes arthritis by exploring the factors revealed in this DR4 tg mouse model to the model of arthritis we have developed in FcγRIIb deficient mice.

Specific Aims:

Studies in DR4 tg mice:

1. Generate antibodies to probe the presence of Cit peptide/protein antigens in the joints of arthritic vs non-arthritic mice.
2. Compare the synovial tissue in the arthritic vs. non-arthritic mice for evidence of apoptotic generation of Cit antigens and shared epitope expression.
3. Examine the arthritogenic properties of Cit-specific immune response from arthritic and non-arthritic DR4 tg mice.
 - 3.1. Compare the arthritogenic properties of Cit-specific antibodies in the different mice
 - 3.2. Compare the arthritogenic properties of Cit-specific immune cells in these different mice.

Studies in human RA:

4. Identify intra-articular targets of human RA Cit peptide (CCP2)-specific antibodies responsible for the development of arthritis in FcγRIIb deficient mice.
5. Compare the arthritogenic properties of Cit Peptide (CCP2)-specific antibodies in those with RA and their unaffected siblings.

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:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen?		Is it known to be an animal pathogen?		Is it known to be a zoonotic agent?		Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level			
	YES/NO	YES/NO	YES/NO	YES/NO	YES/NO	YES/NO			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					
	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 2+	<input type="checkbox"/> 3					

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

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 checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Blood, synovial fluid	Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Blood, spleen, lymph node	2009-101
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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 1 2 2+ 3

Additional Comments: _____

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	From patients and volunteers	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid	Serum from patients	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	No	<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)	No	Not Applicable		Not Applicable

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

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

*** Please attach a plasmid map.*

****No Material Safety Data Sheet is required for the following strains of E. coli:*

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

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.*

4.3.1 Will virus be replication defective? YES NO

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

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 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 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? YES, number: **09684E** NO PENDING

7.0 Animal Experiments

7.1 Will live animals be used? YES NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used **MiceB6, DR4 tg, FcyRIIb def, BALB/C**

7.3 AUS protocol # **2009-101**

7.4 List the location(s) for the animal experimentation and housing. **WV115, DSB 5552**

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

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 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 9.0

8.2 Will live animals be used? YES NO

8.3 If YES, please specify the animal(s) used:

- | | | |
|-----------------------------|--|-----------------------------|
| ◆ Pound source dogs | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Pound source cats | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Cattle, sheep or goats | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Non-human primates | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Wild caught animals | <input type="checkbox"/> YES, species & colony # | <input type="checkbox"/> NO |
| ◆ Birds | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify | <input type="checkbox"/> NO |

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Tetanus Toxoid**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone **Not available, non-hazardous reagent**

9.4 How much of the toxin or hormone is handled at one time*? **25ug**

9.5 How much of the toxin or hormone is stored*? **50ug**

9.6 Will any biological toxins or hormones be used in live animals? YES NO
If **YES**, Please provide details:

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? 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 insect?

10.4 What is the life stage of the insect?

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

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

10.7 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:

11.0 Plants

- 11.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 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.

An X in the check box indicates you agree with the above statement...
Enter Your Name Dr. Ewa Cairns **Date:** Sept. 4 2012

14.0 Containment Levels

14.1 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

14.2 Has the facility been certified by OHS for this level of containment?
 YES, location and date of most recent biosafety inspection: **Nov 2011**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0154**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? YES NO
If **YES** please describe:

15.2 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:
Occupational Health and Safety procedure will be followed. Immediately the wound will be washed with soap and running water for 5 minutes. The wound will be squeezed to induce bleeding if possible. Medical advice will be sought immediately by reporting to Health Services in UCC, Rm 11 or 25. UWO Accident/Incident Form will be filled, signed by supervisor and Faxed (x82079) to UWO H.R. in 24hrs.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html>

An X in the check box indicates you agree with the above statement...
Enter Your Name Dr. Ewa Cairns **Date:** Sept 4, 2012

15.4 Additional Comments: _____

16.0 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:



**LIST BIOLOGICAL
LABORATORIES, INC.**

540 DIVISION STREET ▪ CAMPBELL ▪ CALIFORNIA 95008-6906 ▪ USA
408-866-6363 ▪ 800-726-3213 ▪ FAX 408-866-6364 ▪ EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Product #191A

**CERTIFICATE OF ANALYSIS
TETANUS TOXOID
from *Clostridium tetani*
Lot #19121A1A**

NEW FORMULATION

Contents

Each vial, when reconstituted with 50 μ l of water, contains 25 μ g of tetanus toxoid in 100 mM Histidine, 100 mM NaCl, pH 7.0 and 2% Trehalose. **Handle product gently; do not vortex.**

Concentration

Protein concentration is determined by measuring the absorbance at 280 nm using an extinction coefficient of 1.24 for a concentration of 1 mg/ml.

Toxicity

This product is produced from tetanus toxin by inactivation with formaldehyde. The resulting toxoid is essentially nontoxic. At the highest dose tested (4 μ g/mouse) there were no deaths, no signs of illness, and no spastic paralysis during seven days of observation.

Assay Results

In immunodiffusion studies, tetanus toxoid shows immunoprecipitation when tested with an antiserum specific to tetanus toxoid.

This product has been tested for endotoxin levels and was found to be acceptable.

Packaging and Storage

Tetanus toxoid is supplied as a lyophilized powder, sealed under vacuum. Store at 2-8°C prior to reconstitution. Following reconstitution, aliquot and freeze. Do not exceed one freeze-thaw cycle.

Handling

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended for use when handling lyophilized material.

(continued)

This product is intended for research purposes by qualified personnel only. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.

Production: EM Date: 2/24/12 Management: JL Date: 2/24/12 QA/QC: GR Date: 2/24/12