THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
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).

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. 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

PRINCIPAL INVESTIGATOR	David E. Heinrichs
DEPARTMENT	Microbiology and Immunology
ADDRESS	SDRI 215
PHONE NUMBER	86595
EMERGENCY PHONE NUMBER(S)	519-639-9138
EMAIL	deh@uwo.ca

Location of experimental work to be carried out: Building(s) __SDRI____ Room(s)_209-212-215_____

*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:____CIHR/NSERC_____
GRANT TITLE(S): _____Role of iron and heme binding proteins in_S. aureus pathogenesis_/ S. aureus siderophore synthetase structure/function / Iron metabolism in coagulase-negative staphylococci / S. aureus targets for inhibitor discovery

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

Name	UWO E-mail Address	Date of Biosafety Training
Cristina Marolda	cmarolda@uwo.ca	2006
Federico Beasley	fredbeasley@gmail.com	2006
Johnson Cheung	jcheun56@uwo.ca	2008
Jessica Sheldon	Jsheldo2@uwo.ca	2010
Dustin Kennedy	dkenne@uwo.ca	2010
John Cooper	jcoope23@uwo.ca	2008
Alan Poole	apoole22@uwo.ca	2010
Hanbo Zhang	hzhan59@uwo.ca	2010
Sung Ho Um	sum2@uwo.ca	2010

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Microorganisms are stored at -80°C freezer and upon completion of the experiments they are disposed by autoclaving them. For experiments in the lab, bacteria are grown in flasks or test tubes, or on petri dishes. The minimal volumes of bacteria as required are used.

Please include a one page research summary or teaching protocol.

S. aureus is the leading cause of bacterial infections involving the bloodstream, lower respiratory tract, and skin and soft tissue in many developed countries. This bacterium is especially noteworthy for its ability to acquire antibiotic resistance. Indeed, since its description in the early 1960s, methicillin resistant S. aureus (MRSA) (MRSA is resistant to all available penicillins and other b-lactams) has spread widely and is now endemic in most hospitals worldwide; epidemics and pandemics of antibiotic resistant S. aureus have arisen over the course of the past 60 years. Strikingly, the mortality associated with invasive MRSA infections is approximately 20% and, in the U.S., these infections are the leading cause of death by any infectious agent, surpassing those caused by HIV/AIDS. The success of S. aureus as a leading pathogen is undoubtedly attributed to its extensive repertoire of virulence factors. These virulence factors promote host colonization and dissemination, alter leukocyte recruitment or function, destroy leukocytes, and inhibit complement and antimicrobial peptides. Given the highly virulent nature of S. aureus, combined with antibiotic resistance and the lack of an approved vaccine, there is a clear need for basic science to discover novel therapeutic targets in this bacterium, the foundation of which will be a fundamental understanding of the basic physiology of this bacterium.

Iron plays a decisive role in the infectious disease process and, therefore, proteins involved in iron sensing and iron acquisition are considered important virulence factors. Indeed, the normal host environment encountered by pathogenic microorganisms is iron limiting. ***The central hypotheses in this research program are that the acquisition of iron is essential for in vitro and in vivo growth of S. aureus and, accordingly, that inhibition of iron uptake systems can decrease, or abolish, the virulence of S. aureus.*** *Hence, we have spent the past grant period actively pursuing the identity of iron uptake pathways, and the biochemistry and structure-function relationships of several key iron-siderophore and heme binding proteins. My research group is internationally-recognized for our work in defining the repertoire of iron acquisition systems in S. aureus, including both heme and non-heme uptake systems. Our most recent work has demonstrated that there exists novel and as yet undefined mechanisms of iron acquisition from heme and hemoglobin. These mechanisms almost certainly are involved in augmenting the virulence potential of this pathogen.*

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? x YES O 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? xYES O NO
If YES, please give the name of the species. _Staphylococcus_____
What is the origin of the microorganism(s)? __humans/ animals_____ _____
Please describe the risk (if any) of escape and how this will be mitigated: _____Minimal risk – as a level II laboratory we take all necessary precautions as per safety training, to mitigate risk to our laboratory personnel and the public. Lab is locked throughout the day to limit unnecessary traffic.

Please attach the CFIA permit. A-2010-05214-4
Please describe any CFIA permit conditions:
_____None_____

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
S.aureus	x Yes No	x Yes No	x Yes No	2 L		1 x 2 2+ 3
S. epidermidis	x Yes No	x Yes No	Yes x No	100 ml		1 x 2 2+ 3

S. lugdunensis	yes	Yes	no	100mL		2
S. saprophyticus	yes	yes	no	100mL		2
S. haemolyticus	x Yes No	x Yes No	Yes x No	100 ml		1 x 2 2+ 3
E.coli DH5	Yes x No	Yes x No	Yes x No	6 L		x 1 2 2+ 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 x 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	Yes No		Not applicable
Rodent	Yes No		
Non-human primate	Yes No		
Other (specify)	Yes 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	Yes No			
Rodent	Yes No			
Non-human primate	Yes No			
Other (specify)	Yes 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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? x 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	Blood agar plates	Yes x Unknown		O 1 x 2 2+ O 3
Human Blood (fraction) or other Body Fluid	Transferrin/Haemoglobin (Sigma)	Yes x Unknown		O 1 x 2 2+ O 3
Human Organs or Tissues (unpreserved)		Yes Unknown		O 1 O 2 2+ O 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? xYES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? x 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
E coli	*We use many different plasmids, many of which do not have a map*	*bacteria*	*Bacterial genes*	*None of our manipulations to bacteria or vector would result in increased pathogenicity or be carcinogenic in nature*

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

For your information, attached PDF's are an example of an MSDS and map of plasmid pUC19

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
YES, complete table below x 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 _____ x NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ x NO
- SV 40 Large T antigen YES x NO
- E1A oncogene YES x NO
- Known oncogenes YES, please specify _____ x NO
- Other human or animal pathogen and or their toxins YES, please specify _____ x NO

4.5 Will virus be replication defective? YES xNO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES x 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? x YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used___Mice_____

6.3 AUS protocol # _____2009-041_____

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

6.5 Will the agent(s) be shed by the animal: YES x NO, please justify: internal organs are abscessed by staphylococcal bacteria – organs are extracted for analysis which includes tissue staining and CFU determination

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)? xYES x No If no, please proceed to section 8.0

7.2 Will live animals be used? x 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: _____mice are injected with Staphylococci , which are zoonotic (to our knowledge, they have no endogenous zoonotic hazard_____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____superantigens from staphylococci_and streptococci_____(e.g. TSST-1, SEE, SEB_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.
MSDS for SEB is attached as PDF. There are none for the other toxins as they are not commercially available – we prepare them ourselves from S. aureus culture supernatants.

8.3 What is the LD_{50} (specify species) of the toxin__e.g. TSST-1: ___~75 ug for young rabbits_____

Info taken from recent literature:
Toxic and lethal doses of SEB vary greatly between animal species, mostly because of differences in receptor-binding affinities, and also vary depending on the route of exposure. In humans, the estimated 50% lethal dose (LD_{50}) is 0.02 μg/kg and 50% effective dose (ED_{50}) is 0.0004 μg/kg by aerosolized exposure. No data exist on the LD_{50} and ED_{50} in humans by other routes of exposure. The ED_{50} is estimated to be 0.03–0.26 μg/kg in monkeys and 12–40 μg in chimpanzees, by intraperitoneal or intravenous challenge. The extrapolation of the estimated values of ED_{50} of nonhuman primates to humans would suggest that 2 μg versus 840 μg of SEB would be needed to cause symptoms in a 70-kg person through the ocular or cutaneous route. Occurrence of symptoms in two persons after exposure to dosages of SEB <50 μg provides support that the lower ED_{50} value in monkeys may also apply to humans.

____

8.4 How much of the toxin is handled at one time*? _____5 mg_____

8.5 How much of the toxin is stored*? _____<20 mg_____

8.6 Will any biological toxins be used in live animals? x YES, Please provide details:___this information is included in AUS protocol # 2009-041_____ NO

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

9.0 Insects

9.1 Do you use insects? YES x 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: _____
O "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:

_____

_____

10.0 Plants

10.1 Do you use plants? YES x 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 O "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:

_____

_____

_____

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

_____

_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? xYES, please give country of origin__Usually USA_____
NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? X 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.

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 X 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
X 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): BIO-UWO-0015

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.
None required

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:

Take appropriate action for the event, based on our combined knowledge and safety training, and followup with report to OHS

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: January 27, 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:

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Material Safety Data Sheet(s)

Staphylococcus aureus - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Staphylococcus aureus***

SYNONYM OR CROSS REFERENCE: Staphylococcal diseases, impetigo, toxic shock syndrome, food poisoning, intoxication

CHARACTERISTICS: Gram positive cocci, usually in clusters; coagulase positive; non-spore forming; non-motile; many strains produce exotoxins including staphylococcal enterotoxins A,B,C,D,E, toxic shock syndrome toxin (TSST-1) and exfoliative toxins A, and B

SECTION II - HEALTH HAZARD

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

EPIDEMIOLOGY: Occurs worldwide; particularly in areas where personal hygiene is suboptimal; in hospitals by development of antibiotic-resistant strains

HOST RANGE: Humans; to a lesser extent, warm-blooded animals

INFECTIOUS DOSE: Virulence of strains varies greatly

MODE OF TRANSMISSION: Contact with nasal carriers (30-40% of population); from draining lesions or purulent discharges; spread person-to-person; ingestion of food containing staphylococcal enterotoxin (food may be contaminated by food handlers hands); from mother to neonate during delivery

INCUBATION PERIOD: Variable and indefinite, commonly 4-10 days; disease may not occur until several months after colonization; interval between eating food and onset of symptoms is usually 2-4 hours (30 min to 8 hours)

COMMUNICABILITY: As long as purulent lesions continue to drain or carrier state persists; auto-

infection may continue for the period of nasal colonization or duration of active lesions

SECTION III - DISSEMINATION

RESERVOIR: Human; patients with indwelling catheters or IVs act as reservoirs for nosocomial infections; food borne - occasionally cows with infected udders

ZOONOSIS: Yes - direct or indirect contact with infected animals

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Many strains are multi-resistant to antibiotics and are of increasing importance; methicillin resistant (MRSA) strains have caused major outbreaks world-wide; Vancomycin resistant (VRSA) are being increasingly isolated; sensitivity must be determined for each strain

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, iodine/alcohol solutions, glutaraldehyde, formaldehyde

PHYSICAL INACTIVATION: Organisms are destroyed by heat (moist heat - 121° C for at least 15 min, dry heat - 160-170° C for at least 1 hour; enterotoxins are heat resistant, stable at boiling temperature

SURVIVAL OUTSIDE HOST: Carcass and organs - up to 42 days; floor - less than 7 days; glass - 46 hours; sunlight - 17 hours; UV - 7 hours; meat products - 60 days; coins - up to 7 days; skin from 30 min to 38 days

SECTION V - MEDICAL

SURVEILLANCE: Monitor for skin inflammation if wounded by a sharp instrument; isolation of organism from wound or blood, CSF, urine; isolation of> 10^5 organisms or enterotoxin from suspected food

FIRST AID/TREATMENT: Fluid replacement for food poisoning; in localized skin infections, drain abscesses; antibiotic therapy for severe infections

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 29 reported cases up to 1973 with 1 death

SOURCES/SPECIMENS: Clinical specimens - blood, abcesses, lesion exudates, CSF, respiratory specimens, feces, urine

PRIMARY HAZARDS: Injuries from contaminated sharp instruments; ingestion; aerosols

SPECIAL HAZARDS: Direct contact with open cuts and lesions of skin

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities with cultures or potentially infectious clinical materials

PROTECTIVE CLOTHING: Laboratory coat: gloves when skin contact is unavoidable

OTHER PRECAUTIONS: Thorough handwashing before leaving the laboratory and after handling infectious materials

SECTION VIII - HANDLING INFORMATION

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

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection

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: 2011-02-18

Risk Group Classification for Infectious Agents

Leave feedback about this database (questions, comments or suggestions) >

< Main Riskgroup Page and Definitions
SEARCH: Bacteria > Viruses > Fungi > Parasites >

Bacteria Search Results

Genus: Staphylococcus	**Species:** aureus	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:	2	
Belgium 2004:	2	
Switzerland 2003:	2	including subsp. anaerobius, aureus)
United Kingdom 2004:	2	
Germany 2001:	2	AR
NIH 2002	2	
European Community 2000:	2	
Singapore 2004:	2	Singapore Schedule:
Japan:	2	
Human Pathogen: Yes **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS: http://www.phac-aspc.gc.ca/msds-ftss/msds143e.html		

Genus: Staphylococcus	**Species:** caprae	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus	**Species:** epidermidis	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:	2	
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:	2	AR
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** felis

	Risk Group Level	Notes
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	t
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: No **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** haemolyticus

	Risk Group Level	Notes
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:	2	
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** hyicus

	Risk Group Level	Notes
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:	2	t
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** intermedius

	Risk Group Level	Notes
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:	2	t
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** lugdunensis

	Risk Group Level	Notes
Australia/New Zealand 2002:		
Belgium 2004:		

Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** lutrae

	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	t
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: No **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** pasteuri

	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** saccharolyticus

	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	(Peptococcus saccharolyticus)
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus **Species:** saprophyticus

	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:	2	(subsp. bovis & saprophyticus)
NIH 2002		
European Community 2000:		

European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus	**Species:** saprophyticus subsp. bovis	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	t?
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: No **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus	**Species:** saprophyticus subsp. saprophyticus	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** No **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

Genus: Staphylococcus	**Species:** schleiferi	
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:		
Switzerland 2003:	2	X t (including ubsp. coagulans, schleiferi)
United Kingdom 2004:		
Germany 2001:		
NIH 2002		
European Community 2000:		
Singapore 2004:		Singapore Schedule:
Japan:		
Human Pathogen: No **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

American Biological Safety Association, 1200 Allanson Road, Mundelein, IL 60060-3808
Phone: 1-866-425-1385 (toll free), 847-949-1517 **Fax:** 847-566-4580 **E-mail: info@absa.org**

Table 4.2

pUC19

GenBank Accession #: L09137
See page 160 for ordering information.

Feature	Coordinates	Source
lacZα	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap[R])	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AfeI, AflII, AgeI, AleI, ApaI, AscI, AsiSI, AvrII, BaeI, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, MscI, NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SexAI, SfiI, SgrAI, SnaBI, SpeI, SrfI(x), StuI, StyI, SwaI, TliI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

pUC19 is a small, high-copy number *E. coli* plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and >200 per cell at 42°C (2, 3). The multiple cloning site (MCS) is in frame with the *lacZα* gene, allowing screening for insertions using α-complementation.

pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

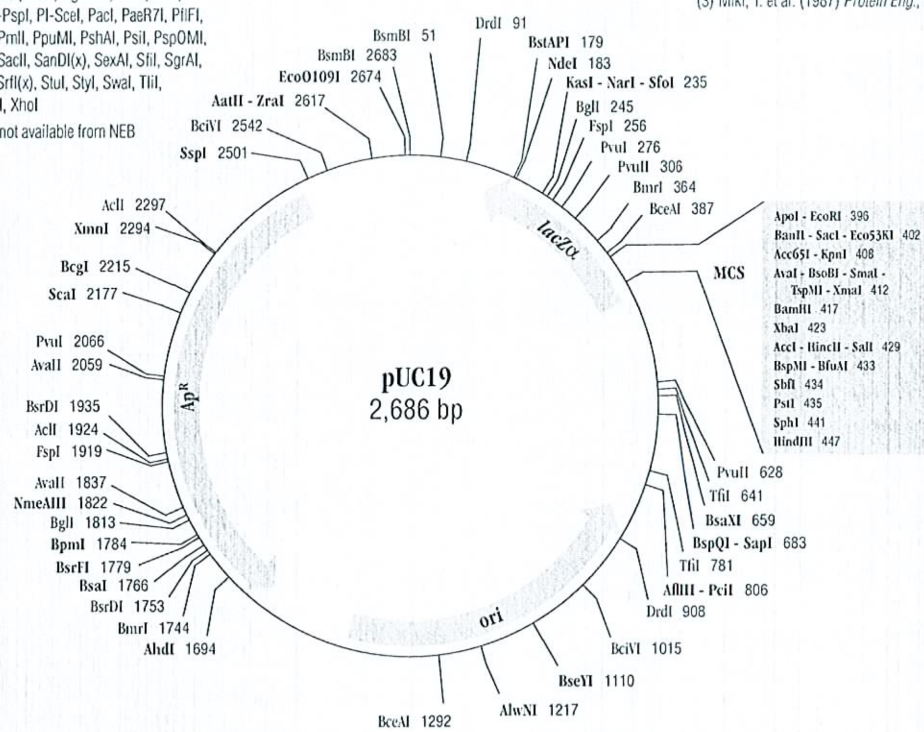
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap[R]) gene coordinates include the signal sequence.

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene*, 33, 103–119.
(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.*, 6, 3385–3393
(3) Miki, T. et al. (1987) *Protein Eng.*, 1, 327–332.



pUC19 MCS

```
        SacI          SmaI          XbaI           SbfI
   EcoRI         KpnI         BamHI          SalI  PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
     .         .         .         .         .         .         .
    400       410       420       430       440       450       460

...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
```

← *lacZα* translational start

pNEB193 MCS

```
        SacI          SmaI    BssHII         PacI                      SbfI
   EcoRI         KpnI          AscI     BamHI           XbaI  SalI   PmeI   PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
     .         .         .         .         .         .         .         .         .         .
    400       410       420       430       440       450       460       470       480       490

...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
```

← *lacZα* translational start

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Fax: (978) 921-1350
Email: info@neb.com
Revision Date: 5/10

MATERIAL SAFETY DATA SHEET (MSDS)

NEB #N3041

PRODUCT NAME:

Product Name: pUC19 Vector Cas.# None

IMPORTANT NOTE:

Per OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005,1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, this product does not require a Material Safety Data Sheet (MSDS). This product does not contain more than 1% of a component classified as hazardous and does not contain more than 0.1% of a component classified as carcinogenic. Therefore New England Biolabs, Inc. does not provide this information on a MSDS. However, when working with this or any chemical reagent, we always recommend the use of gloves, lab coats, and eye protection. New England Biolabs, Inc. assumes no liability for damage resulting from handling or contact with this product.

If you have any questions, please contact info@neb.com.

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 #122

MATERIAL SAFETY DATA SHEET
Enterotoxin type B from *Staphylococcus aureus*

Hazardous Ingredients:

The toxic component is enterotoxin type B, a 28,000 dalton peptide that is secreted by certain pathogenic strains of *Staphylococcus aureus*. On a weight basis, it constitutes approximately 48% of the total mass. The solvent is 0.005 M potassium phosphate, pH 6.8, and represents approximately 52% of the mass.

Physical Properties:

The product is provided as a white lyophilized powder.

Fire and Explosion Hazard Data:

Enterotoxin type B is combustible but not flammable. Use any commercial fire extinguisher.

Health Hazard:

Enterotoxin type B, one of the pathogenic factors produced by certain strains of *S. aureus*, produces vomiting and diarrhea in humans and higher primates. It is estimated that 2-3 ng/kg causes human illness, and this toxin can be lethal at higher doses [Bergdoll, M. (1988) *Meth. Enzymol.* **165**, 324-333]. If contact occurs, flush eyes, skin or wounds thoroughly with water. Seek medical attention.

Reactivity Data:

This product is stable for years in the dried form when stored at 4-7°C. No incompatibilities nor hazardous decomposition products are known. Hazardous polymerization will not occur.

Spill or Leak Procedures:

If a spill occurs, cover with a damp cloth or paper towel. Wipe up and autoclave this material at 121°C and 15 psi for 15 minutes. Further, decontaminate the area with 5% bleach or strong acid.

(continued)

Toxin Info

Special Protection Information:

Wear safety glasses, protective clothing, and rubber or latex gloves. Do not work with this toxin in the dried state; work only with reconstituted material. Avoid inadvertent self inoculation when handling this product in conjunction with hypodermic needles. Do not pipette by mouth. Avoid inhalation of this product.

Special Procedures:

This product is to be used by skilled personnel in a laboratory setting only. Good laboratory technique should be employed. This product is intended for research purposes only. It is not for use in humans and is not to be used as a diagnostic agent.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Enterotoxin type B
Proposed Use Dose:	500 µg
Proposed Storage Dose:	20000 µg
LD $_{50}$ (species):	2000 µg

Calculation:

2000 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 100000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = *10000*

Comments/Recommendations:

Proposed storage dose is over. Please store in two different locations.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	TSST-1
Proposed Use Dose:	500 µg
Proposed Storage Dose:	20000 µg
LD $_{50}$ (species):	75 µg

Calculation:

75 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 3750

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = *375*

Comments/Recommendations:

Proposed storage and use dose is over. LD $_{50}$ of 75 µg is for young rabbits.