THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 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	Dr. Bhagirath Singh
DEPARTMENT	Microbiology & Immunology
ADDRESS	SDRI 224
PHONE NUMBER	83483
EMERGENCY PHONE NUMBER(S)	Ed Lee Chan home: 519-660-8154
EMAIL	bsingh@uwo.ca

Location of experimental work to be carried out: Building(s): SDRI Room(s) 224,225.226,227_

*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
GRANT TITLE(S): Various

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

Name	UWO E-mail Address	Date of Biosafety Training
Ed Lee-Chan	eleechan@uwo.ca	9/16/20006
Olga Krougly	okrougly@uwo.ca	11/08/2006
Enayat Nikoopour	enikpoopo@uwo.ca	11/23/2006

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.

Bacillus Calmette-Guerin (BCG) from Attenuated Strain of Mycobacterium bovis
BCG will be injected ip into mice in the ACVS Dental Sciences Blg. These mice will be monitored for the development of diabetes.
BCG will be stored in a secured 4°C fridge in SDRI 224.
All waste, bedding and carcases will be incinerated. Cages will be chemically treated and autoclave.

Human Source Material

Whole Blood, Synovial Tissue.
The tissue and cells will be handled in a Biological Safety cabinet.
All solid waste will be collected in a biohazard bags and autoclave before disposal.
All liquid waste will be collected and autoclave before disposal.

Synovial Fluid:
The fluid will be handled in a Biological Safety cabinet.
All solid waste will be collected in a biohazard bags and autoclave before disposal.
All liquid waste will be collected and autoclave before disposal.

Rodent Source Material

Liver, Kidney,Lymph Nodes, Spleen, Pancreas, Boon Marrow:
Whole cell will be extracted from these tissues and used in cell cultures experiments and adoptive transfer into mice.

Only Fresh tissues will be used. The cells will be handled in a Biological Safety cabinet.
All solid waste will be collected in a biohazard bags and autoclave before disposal.
All liquid waste will be collected and autoclave before disposal.
All mice that received adoptive cells will be housed at ACVS. All waste, bedding and carcases will be incinerated. Cages will be autoclave.

Biological Toxins

Staphylococcal Enterotoxin B:
Staphylococcus enterotoxin B (SEB) is a bacterial superantigen that binds to MHC class II outside the conventional peptide antigen-binding site. We will be using SEB to determine the competitive binding pattern of Insulin peptide (autoantigen peptide) to MHC class II, which determine the outcome of the immune response leading to autoimmune disorders such as insulin dependent diabetes mellitus (IDDM).
Spleen cells from unimmunized NOD and BALB/c mice will be incubated with various FITC-labeled peptides (Insulin, OVA. CLIP), then washed and incubated with various concentrations of SEB for 1 hour on ice. The binding of FITC-labeled peptides will be measured using flow cytometry.
Staphylococcus enterotoxin B will be stored in a secured 4°C fridge in SDRI 224.
All waste will be collected and stored in biohazard waste bags and autoclave before disposal.

Pertussis Toxin:
Experimental autoimmune encephalomyelitis (EAE) is a model for investigating interactions of Th17 cells, and their functional role in causing and ameliorating autoimmune disease. Pertussis toxin will be injected ip into mice to weaken the blood brain barrier and facilitate rapid induction of EAE.
Pertussis Toxin will be diluted to 1ng/ml and stored in a secured 4°C fridge in SDRI 224.
All waste, bedding and carcases will be incinerated. Cages will be chemically treated and autoclave.

Cells Types

U937, PU5, NIT-1, DC-2.4:
These cell types will be used in cell culture experiments incubated at 37°C.
Stock supply stored in –80°C freezer or Liquid Nitrogen
All solid waste will be collected in a biohazard bags and autoclave before disposal.
All liquid waste will be collected and autoclave before disposal.

DH5α from E.coli will be used in bacterial cloning.
Stock supply stored in –80°C freezer otherwise stored in a 4°C fridge
All waste will be collected and stored in biohazard waste bags and autoclave before disposal.

Please include a one page research summary or teaching protocol.

There are three major area of our research:

a. Cellular basis for the activation of regulatory CD4+CD25+ and effector Th17 T cells in autoimmunity by microbial agents and autoantigens

Regulatory T cells modulate both immunity and autoimmunity. We are exploring the role of these cells in the pathogenesis and prevention of type 1 diabetes (T1D). This includes induction of CD4+CD25+ (Treg) cells and Th17 effector cells. There is reciprocal relationship between Treg cells, which prevent tissue inflammation and promote self-tolerance and Th17 cells that are generally proinflammatory. We are investigating novel IL-17-producing Th17 cell subsets and elucidating the role of proinflammatory cytokines following mycobacterial immunization or after treatment with autoantigens and peptides of autoantigens such as insulin. Our protocol involves using NOD mouse model of T1D and related mouse strains. In some studies peripheral blood cells from diabetic or control non-diabetic subjects may be used in collaborative studies

b. Dendritic cell mediated regulation of T cell mediated autoimmunity in Type I diabetes

Induction and progression of T cell mediated autoimmune diseases such as Type 1 diabetes (T1D) is dependent on antigen presenting cells particularly dendritic cells (DC). Different subsets of DC are critical in the induction and effector phase of the disease. The goal of this project is to use DC in preventing and modulating T1D using animal models. Further, to correlate the data from the mouse model of T1D with human patients, we assess blood DC from subjects with T1D. Additionally, our lab has discovered a novel peptide fragment of apolipoprotein E (ApoE), termed Ep1.B, which induces the differentiation of monocytes into a disease protective DC subset. We also explore mechanisms and disease prevention strategies using regulatory T cell subsets and autoantigens involved in disease. Our protocol involves using NOD mouse model of T1D and related mouse strains. In some studies peripheral blood cells from diabetic or control non-diabetic subjects may be used in collaborative studies

c. Modulation of islet beta cell expansion in pancreatic tissue

There is considerable evidence that insulin producing beta cells in the pancreatic islets these cells can regenerate through formation of new islet-like cell clusters containing beta cells. We previously showed diabetes prevention and islet preservation in NOD mice by treatment with mycobacterail preparations such as complete Freund's adjuvant (CFA) or BCG. Several recent studies have confirmed regeneration of beta cells in the islets and following blocking of autoimmunity. The specific aims of our work is to investigate the expression of various transcription factors of *Reg* family involved in islet beta cell regeneration in pancreas of NOD mice and functionally characterize these cells. The potential regeneration of beta cell regeneration is being investigated to reverse T1D.

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? O YES X 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:

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
E.coli-DH5α	O Yes X No	O Yes X No	O Yes X No	500mls	Gibco/ Invitrogen	X 1 O 2 O 2+ O 3
Bacillus Calmette-Guerin	O Yes X No	O Yes X No	O Yes X No	0.2 ml	Sanofi Pasture	O 1 O 2 X 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 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? X YES O 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	X Yes O No	Blood	Not applicable
Rodent	X Yes O No	Liver, Kidney,Lymph Nodes, Spleen, Pancreas, Boon Marrow	2008-005 Singh
Non-human primate	O Yes X 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)*	Supplier / Source
Human	X Yes O No	U937	ATCC
Rodent	X Yes O No	PU5, NIT-1,DC-2.4	ATCC
Non-human primate	O Yes X 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 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? X YES O 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Whole Blood, Synovial Fluid. LHSC-UH /Canadian Blood Services	O Yes X No O Unknown		O 1 X 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid	Serum, Cells. LHSC-UH	O Yes X No O Unknown		O 1 X 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	Synovial Tissue. LHSC-UH	O Yes X No O Unknown		O 1 X 2 O 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? X YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or transfection
E.coli-DH5α	pGEMEX-1	Promega	Mouse GAD	This mGAD/pGEMEX plasmid with its T7 gene 10 protein expression can now express mouse GAD recombinant protein.

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

4.3 Will genetic modification(s) involving viral vectors be made? O 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 O 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 (NIT-cells) X YES O NO
- E1A oncogene O YES X NO
- Known oncogenes O YES, please specify _____ X NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

4.5 Will virus be replication defective? O YES X NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mice, Rabbits, Rats.

6.3 AUS protocol # 2008-005 SINGH

6.4 Will any of the agents listed in section 4.0 be used in live animals X YES, specify: Adoptive Transfer. O NO

6.5 Will the agent(s) be shed by the animal: O YES X 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)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) Staphylococcal Enterotoxin B, Pertussis Toxin _____
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__
Staphylococcal Enterotoxin B : Human 0.02ug/kg (Emerging Infection Diseases.www.cdc.gov/eid.Vol.10, No.9, Sept. 2004.
Pertussis Toxin: Intravenous-Rat 0.114 mg/kg. (Sigma-Aldrich MSDS)

8.4 How much of the toxin is handled at one time*? Pertussis Toxin : 4 µg per Experiment.
Staphylococcal Enterotoxin B:
Number of mice per experiment: 10-20
Average weight per moues: 20g (0.02kg)
Total Staphylococcal Enterotoxin B handled per experiment: 3-6 ng

8.5 How much of the toxin is stored*? __ Staphylococcal Enterotoxin B: 5mg
Pertussis Toxin: 50 µg

8.6 Will any biological toxins be used in live animals? X YES, Please provide details: See Attach Experimental Description Attach. _____ O 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? O 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? O 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? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? O 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? 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
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? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

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 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 _____

Signature(s)

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. O 1 X 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

X YES, permit # if on-campus BIO-UWO-0066

O NO, please certify

O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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 Western 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 (s)

SIGNATURE _____ Date: _____

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

No additional measures are required for these agents.

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

The person will receive immediate medical attention

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:

Subject: Fwd: Revised Biological Agents Registry Form and Reply to Questions: Singh
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Thu, 09 Dec 2010 15:53:44 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>

-------- Original Message --------
Subject: Revised Biological Agents Registry Form and Reply to Questions: Singh
Date: Thu, 09 Dec 2010 12:48:50 -0800
From: Edwin C Lee-Chan <eleechan@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC:bsingh@uwo.ca

NEW INFO

Comment: 1 Mycobacterium is not spelled correctly on page 2.

Reply: 1 Corrected.

Comment:2 DH5 alpha is E.coli. Stocks should be stored at -80°C, not -20°C.

Reply:2 Corrected. Will store E.Coli at $-80^{o}C$.

Comment:3 BCG is attenuated, not dead.

Reply:3 Understood that BCG is attenuated solution.

Since the answer is "**NO**" to question "**Do you use microorganisms that require a permit from the CFIA?**" We don't need to fill out the rest of this section.

Comment:4

Comment:4 Comment:4 The source of the LD_{50} should be clarified as most sources list a higher LD_{50} for Pertussis toxin.

Reply:4 The source of the LD50 for **Staphylococcal Enterotoxin B** : Human 0.02ug/kg (Emerging Infection Diseases.www.cdc.gov/eid.Vol.10, No.9, Sept. 2004.
See attachment.
The source of the LD50 for **Pertussis toxin** was obtain from Sigma-Aldrich MSDS . This was LD50 for rat Intravenous-Rat 0.114 mg/kg. See attachment

Attach is a full corrected Revised Registry form.

Ed Lee-Chan

Revised 2010 Bioharzad Agent Registry form Dec 13 2010.doc **Content-Type:** application/msword

invitrogen

Material Safety Data Sheet

Revision Date: 20-Sep-2005

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18258
Product name ME DH5A COMPETENT CELLS

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

MSDS'

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	3-7

3. HAZARDS IDENTIFICATION

Emergency Overview

Irritating to eyes. Irritating to skin. Components of the product may be absorbed into the body through the skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects: Eyes. Skin.

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures: Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form: Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C 189	°F No data available
Melting point/range	°C 18.4	°F No data available
Flash point	°C 94	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — Eyes. Skin.

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

Proper shipping name Not classified as dangerous within the meaning of transport regulations

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

PRODUCT MONOGRAPH

ImmuCyst®

Bacillus Calmette-Guérin (BCG), substrain Connaught

Powder for suspension for intravesical use

81 mg

ANTINEOPLASTIC

ATC Code: L03AX03

Sanofi Pasteur Limited
Toronto, Ontario, Canada

Date of Approval:
January 2010

Table of Contents

ImmuCyst®

Bacillus Calmette-Guérin (BCG), substrain Connaught

PART I: HEALTH PROFESSIONAL INFORMATION

SUMMARY PRODUCT INFORMATION

Route of Administration	Dosage Form / Strength	Clinically Relevant Non-medicinal Ingredients
Intravesical Instillation	Powder for suspension: 81 mg (dry weight) equivalent to $10.5 \pm 8.7 \times 10^8$ colony forming units (CFU)	Monosodium glutamate *For a complete listing see DOSAGE FORMS, COMPOSITION AND PACKAGING*

DESCRIPTION

ImmuCyst® [Bacillus Calmette-Guérin (BCG), substrain Connaught] is a freeze-dried preparation made from the Connaught substrain of Bacillus Calmette-Guérin, which is an attenuated strain of *Mycobacterium bovis* for treatment of non-muscle invasive bladder cancer (Ta/T1 papillary tumours and CIS). (1)

The BCG organisms are viable upon reconstitution. The reconstituted product contains $10.5 \pm 8.7 \times 10^8$ colony-forming units (CFU) per instillation dose.

INDICATIONS AND CLINICAL USE

ImmuCyst® is indicated for intravesical use in the treatment of primary or recurrent carcinoma *in situ* (CIS) of the urinary bladder, for prophylaxis of recurrence of CIS of the urinary bladder and for prophylaxis following transurethral resection (TUR) of primary or recurrent stage Ta and/or T1 papillary tumours, or any combination thereof, regardless of antecedent intravesical treatment. (1)

ImmuCyst® is not indicated as an immunizing agent for the prevention of tuberculosis. (1)

CONTRAINDICATIONS

- Known systemic hypersensitivity reaction to any component (see DESCRIPTION and WARNINGS AND PRECAUTIONS) of ImmuCyst® or after previous administration of the medicinal product or a medicinal product containing the same substances.
- Active tuberculosis. Active tuberculosis should be ruled out before starting treatment with ImmuCyst®.
- Current symptoms or previous history of systemic BCG reaction. (See WARNINGS AND PRECAUTIONS.)
- Concurrent febrile illness, urinary tract infection, or gross hematuria. Treatment with ImmuCyst® should be postponed until their resolution. (See WARNINGS AND PRECAUTIONS, Serious and Severe Adverse Events Related Precautions.)
- Congenital or acquired immune deficiencies, whether due to concurrent disease (e.g., AIDS, leukemia and lymphoma) or immunosuppressive therapy (e.g., corticosteroids, cancer therapy [cytotoxic drugs, radiation]) (see DRUG INTERACTIONS, Drug-Drug Interactions) because of the risk of disseminated BCG infection.
- A minimum of 14 days should elapse before ImmuCyst® is administered following biopsy, TUR or traumatic catheterization. (2)

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

Systemic BCG Reactions

A systemic BCG reaction, which may be fatal, is a systemic granulomatous illness, which may occur (although rarely) subsequent to exposure to BCG.

Because it is usually difficult to isolate BCG organisms from affected organs, it is often unclear to what extent such a reaction is caused by an infectious process versus an inflammatory hypersensitivity reaction, hence the term "systemic BCG reaction".

Based on past clinical experience with intravesical BCG, "systemic BCG reaction" may be defined as the presence of any of the following signs, if no other etiologies for such signs are detectable: fever ≥39.5°C for 12 hours; fever ≥38.5°C for 48 hours; pneumonitis; hepatitis; other organ dysfunction outside of the genitourinary tract with granulomatous inflammation on biopsy; or the classical signs of sepsis, including circulatory collapse, acute respiratory distress and disseminated intravascular coagulation. (3) (See ADVERSE REACTIONS.)

Although rare, a systemic BCG reaction is much more likely to occur if ImmuCyst® is administered within 14 days of biopsy, TUR or traumatic bladder catheterization (associated with hematuria).

General

For intravesical instillation only. Do not inject subcutaneously, intradermally or intravenously.

Advice for Patients

Fever, chills, malaise, flu-like symptoms, increased fatigue or an increase in urinary symptoms, (such as burning or pain on urination) can occur. However, patients should be advised to notify their physicians if any of these symptoms last more than 48 hours or increase in severity. Patients should also notify their physicians if they experience any of the following: an increase in urinary symptoms (such as urgency, frequency of urination, blood in urine), joint pain, eye complaints (such as pain, irritation or redness), cough, skin rash, jaundice, nausea or vomiting.

Because ImmuCyst® contains live mycobacteria, excreted urine may also contain live bacteria. Patients should be advised on appropriate infection control procedures to protect family and close contacts from infection. Patients living with or in close quarters to persons who are immunocompromised (on chemotherapy, etc.) should exercise special caution to avoid inadvertently transmitting BCG infection to such susceptible persons. ImmuCyst® is retained in the bladder for as long as possible up to 2 hours and then voided. To avoid transmission of BCG to others, for 6 hours after treatment patients should void while seated to avoid splashing of urine. Urine voided during this time should be disinfected with an equal volume of household bleach for 15 minutes before flushing or disposal. Unless medically contraindicated, patients should be instructed to increase fluid intake to "flush" the bladder for several hours following treatment with ImmuCyst®. Patients may experience burning with the first void after treatment.

Handling Precautions

Handle as infectious. ImmuCyst® contains live attenuated mycobacteria and should be prepared and handled using aseptic technique. (See DOSAGE AND ADMINISTRATION, Reconstitution of Freeze-Dried Product.) BCG infections have been reported in health-care workers preparing BCG for administration.

Nosocomial infections have been reported in immunosuppressed patients receiving parenteral drugs, which were prepared in areas in which BCG was prepared. (4) (5)

Carcinogenesis and Mutagenesis

Mutagenesis and carcinogenesis studies have not been conducted with ImmuCyst® in animals or in humans. Results from clinical trials do not indicate any increased potential for mutagenesis or carcinogenesis although that was not specifically monitored.

Cardiovascular

The risk of ectopic BCG infections has not been determined but is considered to be very small. BCG infection of aneurysms, arterial grafts and cardiac devices can also occur. The benefits of BCG therapy must be carefully weighed against the possibility of ectopic BCG infection in patients with arterial aneurysms or prosthetic devices of any kind.

Genitourinary

Some male genitourinary tract infections (orchitis/epididymitis) have been refractory to multiple drug antimycobacterial therapy and required orchiectomy.

If a bacterial urinary tract infection (UTI) occurs during the course of ImmuCyst® treatment, ImmuCyst® instillation should be withheld until complete resolution of the bacterial UTI, since the combination of a UTI and BCG-induced cystitis may lead to more severe adverse effects on the genitourinary tract; moreover, because BCG bacilli are sensitive to a wide variety of antibiotics; (6) antimicrobial administration may diminish the efficacy of ImmuCyst®.

Hypersensitivity

Acute allergic reaction has been very rarely reported following intradermal injection of BCG vaccine for the prevention of tuberculosis and therefore should be taken into consideration when administering ImmuCyst®.

The stopper of the vial for this product contains natural latex rubber , which may cause allergic reactions.

Immune

For patients with a condition that may in the future require mandatory immunosuppression (e.g., awaiting organ transplant, myasthenia gravis) the decision to treat with ImmuCyst® should be considered carefully.

Treatments using immunosuppressants and/or radiation interfere with the immune response to ImmuCyst® and increase the risk of disseminated BCG infection. (2)

Because of the risk of BCG infection, ImmuCyst® should not be used in immunosuppressed patients or persons with congenital or acquired immune deficiencies, whether due to concurrent disease (e.g., AIDS, leukemia, lymphoma), cancer therapy (e.g., cytotoxic drugs, radiation), or immunosuppressive therapy (e.g., corticosteroids).

Intravesical treatment with ImmuCyst® may induce a positive response to purified protein derivative (PPD). (See DRUG INTERACTIONS.) Determination of a patient's reactivity to PPD should be conducted before administration of ImmuCyst®.

ImmuCyst® should not be handled by persons with an immunologic deficiency.

Peri-operative Considerations

A minimum of 14 days should elapse before ImmuCyst® is administered following biopsy, TUR or traumatic catheterization. There should be no evidence of hematuria prior to instillation of ImmuCyst®.

Sensitivity/Resistance

ImmuCyst® is not sensitive to pyrazinamide. (7)

Serious and Severe Adverse Events Related Precautions

To prevent serious infections, avoid trauma and/or introduction of contaminants to the urinary tract, a minimum of 14 days should elapse before ImmuCyst® is administered following traumatic catheterization. (See CONTRAINDICATIONS.) The treatment schedule should subsequently be resumed as if no interruption in treatment had occurred.

Patients should be monitored for the presence of symptoms and signs of toxicity after each intravesical treatment. If a patient develops persistent fever or experiences an acute febrile illness consistent with BCG infection, BCG instillations should be permanently discontinued, the patient immediately evaluated and treated for BCG infection and an infectious diseases consultation sought. (See CONTRAINDICATIONS.) As standard therapy for BCG infection, treatment with two or more antimycobacterial agents must be initiated promptly while diagnostic evaluation, including cultures, is conducted. Use of single antibiotic therapy is not recommended. Negative cultures do not necessarily rule out infection.

Special Populations

ImmuCyst® is not recommended for prophylactic treatment following TUR of stage TaG1 papillary tumours unless they are judged to be at high risk of tumour recurrence.

In patients with small bladder capacity, increased risk of bladder contracture should be considered in decisions to treat with ImmuCyst®.

Patients undergoing antimicrobial therapy for other infections should be evaluated to assess whether the therapy might diminish the efficacy of ImmuCyst®. (See DRUG INTERACTIONS, Drug-Drug Interactions.)

Pregnant Women

Animal reproduction studies have not been conducted with ImmuCyst®. It is also not known whether ImmuCyst® can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ImmuCyst® should be given to a pregnant woman only if clearly needed. Women should be advised not to become pregnant while on therapy. (1)

Nursing Women

It is not known whether ImmuCyst® can be excreted in human milk. Because many medicinal products are excreted in human milk and because of the potential for serious adverse reactions from ImmuCyst® in nursing infants, it is advisable to discontinue breastfeeding if the mother's condition requires treatment with ImmuCyst®. (1)

Pediatrics

Safety and effectiveness of therapy with ImmuCyst® in pediatric patients has not been established. Therefore, ImmuCyst® should not be used in pediatric patients.

ADVERSE REACTIONS

Adverse event information is derived from clinical trials and worldwide post-marketing experience.

Administration of ImmuCyst® causes an inflammatory response in the bladder and can provoke signs and symptoms of cystitis. (See Table 1 and Table 2.) Such reactions may to some degree be taken as evidence that BCG is evoking the desired response, but careful patient monitoring is required.

Symptoms of bladder irritability are reported in approximately 50% of patients receiving ImmuCyst® and typically begin a few hours after instillation and last 6 - 48 hours. The symptoms are usually seen following the third instillation and tend to increase in severity after each administration. The mechanism of action of the irritative side effects has not been studied, but is most consistent with an immunological

mechanism. There is no evidence that dose reduction or antituberculous drug therapy can prevent or lessen the irritative symptoms of ImmuCyst®. (1) (3)

Clinical Trial Adverse Drug Reactions

Because clinical trials are conducted under very specific conditions the adverse drug reaction rates observed in the clinical trials may not reflect the rates observed in practice and should not be compared to the rates in the clinical trials of another drug. Adverse drug reaction information from clinical trials is useful for identifying drug-related adverse events and for approximating rules.

Description of Data Sources

The adverse reactions which occurred among recipients of ImmuCyst® during clinical trials SWOG 8216 and SWOG 8507 (see ACTION AND CLINICAL PHARMACOLOGY) are listed in Table 1 and Table 2.

Data are categorized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and by decreasing frequency.

Table 1: SWOG Study 8216 - Adverse Reactions (n = 112)

Adverse Reaction	Percent of Patients Overall Induction Plus Maintenance	(Grade ≥3) (Total of 11 Instillations)
Infections and Infestations		
Cystitis	29.5%	(0.0%)
Urinary Tract Infection	17.9%	(0.0%)
Pulmonary Infection	2.7%	(0.0%)
Systemic Infection	2.7%	(1.8%)
Infection	0.9%	(0.9%)
Blood and Lymphatic System Disorders		
Anemia	20.5%	(0.0%)
Leukopenia	5.4%	(0.0%)
Coagulopathy/Thrombocytopenia	0.9%	(0.0%)
Metabolism and Nutrition Disorders		
Anorexia	10.7%	(0.0%)
Nervous System Disorders		
Headache	1.8%	(0.0%)
Dizziness	0.9%	(0.0%)
Cardiac Disorders		
Cardiac (Unclassified)	2.7%	(0.0%)
Gastrointestinal Disorders		
Nausea/Vomiting	16.1%	(0.0%)
Diarrhea	6.3%	(0.0%)
Abdominal Pain	2.7%	(0.0%)
Constipation	0.9%	(0.0%)
Hepatobiliary Disorders		
Liver Involvement	2.7%	(0.0%)
Skin and Subcutaneous Tissue Disorders		
Skin Rash	1.8%	(0.0%)

Musculoskeletal and Connective Tissue and Bone Disorders		
Arthralgia/Myalgia/Arthritis	7.1%	(0.9%)
Flank Pain	0.9%	(0.0%)
Renal and Urinary Disorders		
Dysuria	51.8%	(3.6%)
Urinary Frequency	40.2%	(1.8%)
Hematuria	39.3%	(7.1%)
Urinary Urgency	17.9%	(0.9%)
Renal Toxicity (NOS)	9.8%	(1.8%)
Urinary Incontinence	6.3%	(0.0%)
Bladder Cramps/Pain	6.3%	(0.0%)
Contracted Bladder	5.4%	(0.9%)
Tissue in Urine	0.9%	(0.0%)
Ureteral Obstruction	0.9%	(0.0%)
Reproductive System and Breast Disorders		
Genital Pain	9.8%	(0.0%)
General Disorders and Administration Site Conditions		
Malaise	40.2%	(1.8%)
Fever	38.4%	(2.7%)
Chills	33.9%	(2.7%)
Fatigue	0.9%	(0.0%)

Table 2: SWOG Study 8507 - Adverse Reactions

Adverse Reaction	Percent of Patients			
	Induction (n = 587/587) 6 Instillations		Induction + Maintenance (n = 247/587) 6 + 21 Instillations	
	Overall	(Grade ≥3)	Overall	(Grade ≥3)
Infections and Infestations				
Urinary Tract Infection	1.0%	(0.0%)	4.5%	(0.4%)
Systemic Infection	0.9%	(0.5%)	0.4%	(0.4%)
Pulmonary Infection	0.5%	(0.2%)	NR*	NR
Infection	0.3%	(0.0%)	NR	NR
Cystitis	0.2%	(0.0%)	2.0%	(0.4%)
Blood and Lymphatic Disorders				
Anemia	0.7%	(0.0%)	NR	NR
Leukopenia	0.3%	(0.0%)	NR	NR
Coagulopathy/Thrombocytopenia	0.2%	(0.2%)	NR	NR
Metabolism and Nutrition Disorders				
Anorexia	4.6%	(0.3%)	7.7%	(0.4%)
Nervous System Disorders				
Headache	0.3%	(0.0%)	0.4%	(0.0%)
Dizziness	0.2%	(0.0%)	NR	NR

Cardiac Disorders				
Cardiac (Unclassified)	0.3%	(0.0%)	1.2%	(0.0%)
Gastrointestinal Disorders				
Nausea/Vomiting	2.6%	(0.3%)	4.9%	(0.8%)
Diarrhea	0.9%	(0.0%)	1.2%	(0.4%)
Abdominal Pain	0.3%	(0.0%)	NR	NR
Constipation	NR	NR	0.8%	(0.0%)
Mucositis/Ulcers/Stomatitis	0.2%	(0.0%)	NR	NR
Hepatobiliary Disorders				
Liver Involvement	0.3%	(0.2%)	2.0%	(0.0%)
Granulomatous Hepatitis	0.2%	(0.2%)	NR	NR
Skin and Subcutaneous Tissue Disorders				
Skin Rash	0.7%	(0.3%)	1.2%	(0.0%)
Hypersensitivity Reaction Skin	NR	NR	0.4%	(0.4%)
Skin Abscess	NR	NR	0.4%	(0.0%)
Musculoskeletal and Connective Tissue Disorders				
Arthralgia/Myalgia/Arthritis	0.3%	(0.0%)	1.2%	(0.4%)
Renal and Urinary Disorders				
Dysuria	26.4%	(1.7%)	45.8%	(8.9%)
Hematuria	18.6%	(3.6%)	28.3%	(7.3%)
Urinary Frequency	14.1%	(1.7%)	34.0%	(7.3%)
Urinary Urgency	3.2%	(0.3%)	12.2%	(2.8%)
Bladder Cramps/Pain	1.4%	(0.3%)	3.6%	(1.2%)
Urinary Incontinence	0.9%	(0.3%)	2.0%	(0.8%)
Renal Toxicity	0.9%	(0.0%)	0.8%	(0.0%)
Contracted Bladder	0.5%	(0.2%)	3.6%	(2.0%)
Ureteral Obstruction	0.2%	(0.2%)	NR	NR
Tissue in Urine	NR	NR	0.8%	(0.0%)
Reproductive System and Breast Disorders				
Genital Pain	0.3%	(0.0%)	NR	NR
General Disorders and Administration Site Conditions				
Fever	17.2%	(0.3%)	31.12%	(2.0%)
Malaise	16.7%	(0.7%)	24.7%	(2.0%)
Chills	14.1%	(0.9%)	31.6%	(2.0%)
Fatigue	1.0%	(0.3%)	0.8%	(0.0%)

* NR = Not Reported

Data from Post-Marketing Experience

The following additional adverse events have been spontaneously reported during the post-marketing use of ImmuCyst® worldwide. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to product exposure. Decisions to include these events in labeling were based on one or more of the following factors: 1) severity of the event, 2) frequency of reporting or 3) strength of causal connection to ImmuCyst®.

Data are categorized by MedDRA system organ class.

Infections and Infestations

BCG Infection (rare): BCG is capable of dissemination when administered by the intravesical route. Serious infections, including sepsis with associated mortality, have been reported. BCG infections have also been reported in eye, lung, liver, bone, bone marrow, kidney, regional lymph nodes, peritoneum, genitourinary tract (orchitis/epididymitis) and prostate (e.g., granulomatous prostatitis).

BCG infection of aneurysms and prosthetic devices (including arterial grafts, cardiac devices and artificial joints) has also been reported. (8) (9)

Joint symptoms (arthritis, arthralgia), ocular symptoms (including conjunctivitis, uveitis, iritis, keratitis, granulomatous choreoretinitis), urinary symptoms (including urethritis), skin rash, alone or in combination (Reiter's syndrome), have been reported following administration of ImmuCyst®. For the reports of Reiter's syndrome, the risk seems to be more elevated among patients who are positive for HLA-B27. (10)

Renal abscess (very rare).

Respiratory, Thoracic and Mediastinal Disorders

Pneumonia, interstitial lung disease.

Skin and Subcutaneous Tissue Disorders

Erythema nodosum.

Renal and Urinary Disorders

Renal failure, pyelonephritis, nephritis (including tubulointerstitial nephritis, interstitial nephritis and glomerulonephritis).

Urinary retention (including bladder tamponade and feeling of residual urine).

General Disorders and Administration Site Conditions

Flu like symptoms (rare).

Investigations (Laboratory Tests)

Abnormal/increased blood creatinine or blood urea nitrogen (BUN).

Physicians, nurses and pharmacists should report any adverse reaction related to the administration of the product to the appropriate health authorities in accordance with local requirements and to the Global Pharmacovigilance Department, Sanofi Pasteur Limited, 1755 Steeles Avenue West, Toronto, ON, M2R 3T4 Canada. 1-888-621-1146 (phone) or 416-667-2435 (fax). The report should include details of the treatment history with ImmuCyst®, relevant medical history, the symptoms and signs of the adverse reaction, the treatment administered for the reaction and the response to such treatment.

DRUG INTERACTIONS

Serious Drug Interactions

Immunosuppressive Treatments

Treatment combinations using immunosuppressants and/or radiation interfere with the immune response to ImmuCyst® and increase the risk of disseminated BCG infection. (See WARNINGS AND PRECAUTIONS.) (2)

Drug-Drug Interactions

Intravesical treatment with ImmuCyst® may induce a positive response to PPD, which may complicate future interpretations of skin test reactions to PPD when used to diagnose suspected mycobacterial infections. Determination of a patient's reactivity to PPD should be conducted before administration of ImmuCyst®.

Antibacterial Drugs

Antimicrobial therapy for other infections may interfere with the effectiveness of ImmuCyst®. (6) Patients undergoing antimicrobial therapy should be evaluated to assess whether the therapy might diminish the efficacy of ImmuCyst®.

Antituberculosis Drugs

Antituberculosis drugs should not be used prophylactically to prevent the local, irritative side effects of ImmuCyst®. There are no data to suggest that the acute, local urinary tract symptoms common with intravesical BCG are due to mycobacterial infection.

ImmuCyst® is not sensitive to pyrazinamide. (7)

DOSAGE AND ADMINISTRATION

Recommended Dose

One dose of ImmuCyst® consists of the intravesical instillation of 81 mg BCG.

Intravesical treatment of the urinary bladder should begin a minimum of 14 days after biopsy or TUR (see CONTRAINDICATIONS and WARNINGS AND PRECAUTIONS) and consists of induction and maintenance therapy.

- The induction therapy schedule consists of one intravesical instillation of ImmuCyst® each week for 6 weeks for a total of 6 doses.
- Based on clinical studies performed with ImmuCyst®, maintenance therapy following induction is highly recommended. After a 6-week pause, one intravesical dose should be given each week for 1 to 3 weeks. Then, one dose should be given each week for 1 to 3 weeks at 6, 12, 18, 24, 30 and 36 months following the initiation of induction treatment.

Reconstitution of Freeze-Dried Product

The preparation of ImmuCyst® should be done using **aseptic technique**. A separate area for the preparation of the ImmuCyst® suspension is recommended in order to avoid cross contamination. The person responsible for mixing the agent should wear gloves, eye protection, a mask and gown to avoid inhalation of BCG organisms and inadvertent exposure of broken skin to BCG organisms.

When handling and reconstituting ImmuCyst®, care should be taken so as to avoid needle stick injuries.

ImmuCyst® should not be handled by persons with an immunologic deficiency. (See WARNINGS AND PRECAUTIONS.)

Do not remove the rubber stopper from the vial.

Prepare the surface of the ImmuCyst® and diluent (if provided) vials using a suitable antiseptic.

Presentation with diluent: Using a 5 mL sterile syringe and needle, draw into the syringe a volume of air equal to the volume of diluent in the vial. Pierce the center of the rubber stopper in the vial containing diluent with the sterile needle of the syringe, invert the vial and slowly inject into it the air contained in the syringe. Keeping the point of the needle immersed in the diluent, withdraw into the syringe 3.0 mL of

diluent for the 81 mg vial presentation. Then, holding the syringe-plunger steady, withdraw the needle from the vial.

Presentation without diluent: Using a 5 mL sterile syringe and needle, draw up 3 mL of sterile preservative-free saline solution.

For both presentations: Using the same syringe and needle, pierce the rubber stopper in the vial of freeze-dried material with the needle. Hold the vial of freeze-dried material upright and pull the plunger of the syringe back to create a mild vacuum in the vial. Release the plunger and allow the vacuum to pull the saline from the syringe into the vial of freeze-dried material. After all the saline has passed into the freeze-dried material, remove the needle and syringe.

Shake the vial gently until a fine, even suspension results. Avoid foaming since this will prevent withdrawal of the proper dose. Withdraw the entire contents of the reconstituted material from the vial into the same 5 mL syringe. Return the vial to an upright position before removing the syringe from the vial.

Further dilute the reconstituted material from the vial (1 dose) in an additional 50 mL of sterile, preservative-free saline to a final volume of 53 mL for intravesical instillation.

Any reconstituted product which exhibits flocculation or clumping that cannot be dispersed with gentle shaking should not be used.

Administration

For intravesical instillation only. Do not inject subcutaneously or intravenously.

This dose is prepared by reconstituting 1 vial containing 81 mg freeze-dried BCG with 3 mL of diluent or with 3 mL of sterile, preservative-free saline. The reconstituted BCG is further diluted in 50 mL of sterile, preservative-free saline, for a total of 53 mL instillation volume. (See WARNINGS AND PRECAUTIONS and DOSAGE AND ADMINISTRATION, Reconstitution of Freeze-Dried Product.)

A urethral catheter is inserted into the bladder under **aseptic conditions**, the bladder is drained and then the 53 mL suspension of ImmuCyst® is instilled slowly by gravity, following which the catheter is withdrawn.

The patient retains the suspension for as long as possible up to two hours. The patient should lie prone for the first 15 minutes following instillation. Thereafter, the patient is allowed to be up. At two hours after the instillation, all patients should void in a seated position for hygienic safety reasons. (See WARNINGS AND PRECAUTIONS and SPECIAL HANDLING INSTRUCTIONS.) Unless medically contraindicated, patients should be instructed to increase fluid intake in order to flush the bladder in the hours following BCG treatment.

OVERDOSAGE

Not documented.

ACTION AND CLINICAL PHARMACOLOGY

Pharmacodynamics

When administered intravesically as a cancer therapy, BCG promotes a local acute inflammatory and sub-acute granulomatous reaction with macrophage and leukocyte infiltration in the urothelium and lamina propria of the urinary bladder. (11) (12) The local inflammatory effects are associated with an elimination or reduction of non-muscle invasive cancerous tumours of the urinary bladder (Ta/T1 papillary tumours and CIS). The exact mechanism of action is unknown, but the anti-tumour effect appears to be T-lymphocyte dependent. (12) (13)

Pharmacokinetics

Because ImmuCyst® contains live mycobacteria, excreted urine may also contain live bacteria. (See WARNINGS AND PRECAUTIONS and SPECIAL HANDLING INSTRUCTIONS.)

STORAGE AND STABILITY

ImmuCyst® should be stored at 2° to 8°C (35° to 46°F) (i.e., in a refrigerator). It should not be used after the expiration date marked on the vial, otherwise it may be inactive.

At no time should the freeze-dried ImmuCyst® be exposed to direct or indirect sunlight. Exposure to artificial light should also be kept to a minimum.

Reconstituted Product

Once reconstituted, the product should be used immediately.

Reconstituted product should not be exposed to direct or indirect sunlight. Exposure to artificial light should also be kept to a minimum.

If there is an unavoidable delay between reconstitution and administration, this delay should not exceed 2 hours at a temperature between 2° to 25°C (35° to 77°F).

Any reconstituted product, which exhibits flocculation or clumping that cannot be dispersed with gentle shaking, should not be used.

SPECIAL HANDLING INSTRUCTIONS

Instructions for Disposal

Unused product, packaging and all equipment and materials used for instillation of the product (e.g., syringes, catheters) should be placed immediately in a container for biohazardous materials and disposed of according to local requirements applicable to biohazardous materials.

Urine voided during the 6-hour period following ImmuCyst® instillation should be disinfected with an equal volume of 5% hypochlorite solution (undiluted household bleach) and allowed to stand for 15 minutes before flushing. (See WARNINGS AND PRECAUTIONS.)

DOSAGE FORMS, COMPOSITION AND PACKAGING

Dosage Forms

ImmuCyst® is supplied as a sterile lyophilized white powder in a vial containing 81 mg. If provided, the diluent is a sterile clear colourless solution supplied in a vial containing 3 mL.

Composition

Active Ingredients:

Bacillus Calmette-Guérin (BCG), substrain Connaught	81 mg

Other Ingredients:

Excipient

Monosodium glutamate	150 mg (5% w/v)

Diluent (if provided):

Sodium chloride	25.5 mg (0.85% w/v)
Disodium hydrogen phosphate	7.5 mg (0.25% w/v)
Sodium dihydrogen phosphate	1.7 mg (0.06% w/v)
Polysorbate 80	0.75 mg (0.025% w/v)
Water for injection	up to 3 mL

No preservative is added.

Packaging:

ImmuCyst® is supplied in a package containing either:

- one 81 mg vial of BCG with one 3 mL vial diluent
- one 81 mg vial of BCG

ImmuCyst® is supplied in an amber Type 1 glass vial and the diluent (if provided) is supplied in a clear Type 1 glass vial. The stopper for both vials contains natural latex rubber.

Vaccine Information Service: 1-888-621-1146 or 416-667-2779.
Business Hours: 8 a.m. to 5 p.m. Eastern Time Monday to Friday.
Full product monograph available on request or visit us at www.sanofipasteur.ca

Product information as of January 2010.

Manufactured by:
Sanofi Pasteur Limited
Toronto, Ontario, Canada

R11-0110 Canada

PART II: SCIENTIFIC INFORMATION

PHARMACEUTICAL INFORMATION

Product Characteristics

ImmuCyst® [Bacillus Calmette-Guérin (BCG), substrain Connaught] is prepared from a culture of the Connaught strain of Bacillus Calmette-Guérin (BCG), which is an attenuated strain of living bovine tubercle bacillus, *Mycobacterium bovis*. The bacilli are lyophilized and are viable upon reconstitution.

The reconstituted product contains $10.5 \pm 8.7 \times 10^8$ colony-forming units (CFU) per instillation dose when resuspended. (1)

ImmuCyst® is supplied in a single vial containing 81 mg of BCG with a 3 mL vial of diluent (if provided). The product and the diluent (if provided) contain no preservative. One dose consists of one 81 mg vial of reconstituted material further diluted in 50 mL sterile, preservative-free saline.

CLINICAL TRIALS

Study Demographics and Trial Design

Table 3: Summary of Patient Demographics for Clinical Trials

Study #	Trial design	Dosage, route of administration and duration	Study subjects (n=number)	Mean age (Range)	Gender
SWOG 8216	Randomized	81 mg intravesically	127	65-68	male
SWOG 8507	Randomized	81 mg intravesically	389	62-73	male & female

Clinical studies have proven the effectiveness of ImmuCyst® for patients with non-muscle invasive bladder cancer at the Carcinoma *in situ* (CIS), Ta and T1 stages, including two multicentre controlled, randomized trials.

In the first study SWOG 8216, ImmuCyst® was compared to doxorubicin hydrochloride (Adriamycin®) among patients with either CIS or recurrent papillary tumours or both. (14) ImmuCyst® was administered intravesically once each week for 6 weeks, with an additional single instillation at 3, 6, 12, 18 and 24 months following the initiation of treatment (total of 11 instillations). Doxorubicin was administered once each week for 5 weeks, with an additional 11 single monthly treatments.

For patients with CIS, the complete response rate (i.e., negative biopsies and urine cytology) within 6 months of the initiation of treatment was 70% with ImmuCyst® versus 34% with doxorubicin ($p < 0.001$); the probability of being disease-free (i.e., having no evidence of bladder cancer) at 5 years was 45% (n = 64 patients) and 18% (n = 67 patients), respectively ($p < 0.001$ by proportional hazards regression model); and among complete responders, the median time to treatment failure was 39 months versus 5.1 months, respectively. Among patients with papillary tumours (Ta or T1) without CIS, the probability of being disease-free at 5 years was 37% (n = 63 patients) with ImmuCyst® versus 17% (n = 68 patients) with doxorubicin ($p = 0.015$ by proportional hazards regression model). (14)

In the second study SWOG 8507, two treatment regimens of ImmuCyst® were compared among similar patients to the first study. (15) (16) The initial study report covered a median follow-up period of 3.2 years (1992), (15) and a recent analysis reported a total of ten years of median follow-up data (2000). (16) A 6-

week induction course alone (total of 6 instillations) was compared to a more intensive regimen consisting of the following: an induction course of one treatment each week for 6 weeks; after a 6-week pause, another treatment each week for 3 weeks; and then maintenance therapy consisting of one instillation each week for 3 weeks at 6 months after the initiation of the induction course and then every 6 months for 36 months (total of 27 instillations from the start of therapy).

Comparing the maintenance regimen to the no-maintenance regimen (i.e., 6-week induction course only), the following results were found: the five-year survival was 78% in the no-maintenance compared to 83% in the maintenance arm (p = 0.08).

The overall five-year recurrence free survival was 41% in the no-maintenance group and 60% in the maintenance group (p <0.0001). The recurrence free survival in the 3-week maintenance group (n = 192 patients) was found to be twice as long as (77 versus 36 months) for the no-maintenance group (n = 192 patients). Among a total of 278 eligible patients with CIS, the complete response rate was increased from expected 68% to 84%. The between arm difference for the overall rate of CIS response was significant at p = 0.004. Among the patients with papillary tumours (Ta or T1) without CIS, the median recurrence free survival was 78 months in the maintenance group (n = 128 patients) and 28 months in the no-maintenance group (n = 126 patients).

This study provides evidence that the 3-week, 3-year BCG maintenance schedule provides superior protection from disease recurrence and improves long-term survival. (15) (16)

TABLE 1: COMPARATIVE STUDIES ON EFFICACY OF IMMUCYST®: TREATMENT REGIMENS AND COMPLETE RESPONSE RATES

Treatment Arm	TREATMENT REGIMEN																Total No. of Instillations	RESULTS		
	Number of Weekly Instillations at Time (in Months) Commencing with the First Instillation i.e., Time 0 = Time of First Instillation.																	CIS Patients with Complete Response		
	0	2	3	4	5	6	7	8	9	10	11	12	18	24	30	36		n	%	p
ImmuCyst®	6	-	1	-	-	1	-	-	-	-	-	1	1	1	-	-	11	64	70	p < 0.001*
versus Doxorubicin[4]	5	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	16	67	34	
ImmuCyst® Maintenance	6	-	3	-	-	3	-	-	-	-	-	3	3	3	3	3	27	97	84	p = 0.004
versus ImmuCyst® Induction Only[6]	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	79	68	

* within 6 Months of Initiation of Treatment

PHARMACOLOGY

When administered intravesically as a cancer therapy, BCG promotes a local acute inflammatory and sub-acute granulomatous reaction with macrophage and leukocyte infiltration in the urothelium and lamina

propria of the urinary bladder. (11) (12) The local inflammatory effects are associated with an elimination or reduction of non-muscle invasive cancerous tumours of the urinary bladder (Ta/T1 papillary tumours and CIS). The exact mechanism of action is unknown, but the anti-tumour effect appears to be T-lymphocyte dependent. (12) (13)

General Discussion of BCG Therapy for Bladder Cancer

CIS of the Urinary Bladder

CIS may occur either alone or in association with papillary tumours, particularly those of higher grade. CIS may be multifocal and may be also associated with multifocal pre-malignant dysplastic lesions. While transurethral resection (TUR) is the primary treatment for CIS, it is often not curative: some lesions may be either undetectable or unresectable or both. Furthermore, even with curative TUR, CIS is associated with a high incidence of recurrence and of recurrence of higher-stage lesions, including cancer invasive of the muscle layer of the urinary bladder (stage T2 or higher). Intravesical ImmuCyst® [Bacillus Calmette-Guérin (BCG), substrain Connaught] has been studied and established as both an alternative to radical surgical treatment for CIS and as prophylaxis for recurrence of CIS.

Papillary Tumours of the Urinary Bladder

While TUR is the primary treatment of non-muscle invasive papillary tumours (Ta/T1 tumours), these tumours have a tendency to recur and to progress. This is particularly true when there are two or more co-existing papillary tumours, when there has already been a recurrence of such tumours, or when there is co-existing CIS. In these circumstances, ImmuCyst® has been shown to increase significantly the time to recurrence when administered intravesically for prophylactic purposes following TUR.

TOXICOLOGY

Data from animal studies do not suggest any special hazards other than those already reported from human studies. (1)

Vaccine Information Service: 1-888-621-1146 or 416-667-2779.
Business hours: 8 a.m. to 5 p.m. Eastern Time Monday to Friday.
Full product monograph available on request or visit us at www.sanofipasteur.ca

Product information as of January 2010.

Manufactured by:
Sanofi Pasteur Limited
Toronto, Ontario, Canada

R11-0110 Canada

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IMPORTANT: PLEASE READ BEFORE TAKING THIS MEDICATION

PART III: CONSUMER INFORMATION
ImmuCyst®

Bacillus Calmette-Guérin (BCG), substrain Connaught

This leaflet is Part III of a three-part "Product Monograph" published when ImmuCyst® was approved for sale in Canada. It gives Consumers information about ImmuCyst®. Because this is a summary, it does not tell you everything about the medication. Contact your doctor or pharmacist if you have any questions about this product.

ABOUT THIS MEDICATION

What the medication is used for:

ImmuCyst® is used to treat cancerous growths (tumours) on the surface of the bladder. ImmuCyst® also prevents the growth of new tumours.

What it does:

ImmuCyst® works by stimulating your body's immune system to fight against bladder tumours. This form of treatment is called immunotherapy. Research has shown that immunotherapy is a more effective treatment than chemotherapy in fighting the growth of bladder tumours.

When it should not be used:

- If you get a bacterial urinary tract infection (UTI) while you are taking ImmuCyst® or if you have large amounts of blood in your urine, your doctor may stop the treatment.
- The effects of ImmuCyst® on pregnancy are not known. Female patients should use birth control while on ImmuCyst®. Tell your doctor immediately if you think you may be pregnant.
- Women should not breastfeed while on ImmuCyst®.
- Do not use ImmuCyst® if you are allergic to any ingredient in the product.
- People who have active tuberculosis (TB) should not have this treatment.
- People who have any form of immune deficiency should not have this treatment. Immune deficiency may result from diseases (such as AIDS, leukemia and lymphoma) or from treatments that suppress the immune system (such as corticosteroid or cancer therapy that includes cytotoxic drugs or radiation).
- If you have a bladder operation, you should wait a minimum of 14 days before starting to take ImmuCyst®.

What the medicinal ingredient is:

ImmuCyst® is a freeze-dried preparation made from weakened bacteria called *Mycobacterium bovis*. The BCG organisms are alive but weakened.

What the important non-medicinal ingredients are:

Monosodium glutamate: 150 mg

The non-medicinal ingredients of the diluent (if provided) are:
Sodium chloride
Disodium hydrogen phosphate
Sodium dihydrogen phosphate
Polysorbate 80
Sterile water for injection

For a full listing of non-medicinal ingredients see Part I of the Product Monograph

What dosage forms it comes in:

Every vial contains 81 mg (dry weight) of BCG powder. The powder must be mixed with saline. A health-care provider will give you the medication through a catheter (a tube) inserted into your bladder.

Serious Warnings and Precautions

Systemic BCG Reactions

A systemic BCG reaction is a general body illness caused by spread of BCG beyond the bladder or an unusual reaction of your body to BCG within your bladder. This reaction is rare but may occur after ImmuCyst® treatment.

Contact your doctor if you have any of the following symptoms, after having an ImmuCyst® treatment:

- fever higher than 39.5°C for 12 hours
- fever higher than 38.5°C for 48 hours
- difficulty breathing
- skin or eyes turning yellow
- unusual bleeding or bruising

WARNINGS AND PRECAUTIONS

Before you use ImmuCyst® talk to your doctor or pharmacist if you:

- have blood in your urine or a urinary tract infection,
- have had a treatment or a disease (such as AIDS) that weakens your immune system,
- have had radiation therapy for cancer,
- are pregnant, breast feeding or intending to become pregnant during therapy,
- have a major medical or surgical procedure scheduled during or shortly after ImmuCyst® treatment,
- have any allergies to the ingredients in ImmuCyst®.

INTERACTIONS WITH THIS MEDICATION

Treatment using immunosuppressants and/or radiation interfere with the body's response to ImmuCyst®. They also increase the risk of side effects from the medication.

Antibiotic therapy used for other infections may interfere with the effectiveness of ImmuCyst®.

Treatment with ImmuCyst® may cause a positive tuberculosis skin test. The results of a skin test for tuberculosis any time after treatment with ImmuCyst® may show that you have tuberculosis even if you don't. If you need a TB skin test, it should be done before you start treatment with ImmuCyst®.

PROPER USE OF THIS MEDICATION

Before Your Treatment

- Tell your doctor about any medications you take regularly. Certain drugs affect how ImmuCyst® works (e.g., some antibiotics, medications that may suppress your bone marrow or immune system and/or radiation).
- Do not drink fluids for at least 2 hours before your treatment so that your bladder will be empty.
- You will have to go to your doctor's office or the hospital for treatment with ImmuCyst®. The treatment does not take a long time, but you should take the day off because of the things you need to do after your treatment.

Things to Know About Your Treatment

When will you have it?

- Your treatment should begin about 2 weeks after biopsy or transurethral resection.
- For the first course of treatment, you will get one dose of ImmuCyst® into your bladder once-a-week for 6 weeks.
- If your doctor prescribes maintenance treatments, you will continue with one dose per week for 3 weeks after six weeks have gone by since you completed your first course of treatment. After six months have passed since you began your first course of treatment, you will have one dose per week for 1 to 3 weeks every 6 months. Your doctor will decide how long you will need maintenance treatment.

What will they do?

- Your doctor or nurse will place a catheter (tube) into your bladder. If there is any urine in your bladder it will be drained through the catheter.
- The doctor or nurse will attach a container of ImmuCyst® solution to the catheter. The solution will run into your bladder. This process is called instillation.
- When all the solution is in your bladder, the catheter will be removed.

What do you have to do?

- Be sure to lie on your stomach for the first 15 minutes after the catheter is removed. After that, you can get up and move around. This will make sure ImmuCyst® has completely covered the inside of your bladder.
- You must hold the ImmuCyst® inside your bladder for as long as possible, up to 2 hours. After 2 hours, you can empty your bladder.

After Your Treatment

- Unless your doctor tells you not to, you should drink lots of liquids for the next 24 hours. Try to drink at least twelve 250 mL (8 oz.) glasses of liquid per day. Urinate frequently.
- Because ImmuCyst® may be infectious; you should disinfect the urine in the toilet before you flush. To do this, pour one cup of pure undiluted bleach into the toilet bowl every time you urinate. Leave bleach in the toilet bowl for 15 minutes before flushing. You should do this every time you urinate for the first 6 hours after treatment.

SIDE EFFECTS AND WHAT TO DO ABOUT THEM

Some people have unpleasant side effects during their treatment with ImmuCyst®. However, the side effects are usually easy to manage. On your treatment days, they may be worse but they will get better in a few days. It is important for you to stay on ImmuCyst® for the whole treatment time. Completing the treatment helps to prevent the tumour from coming back.

Please talk to your doctor about any side effects that you feel may prevent you from finishing the treatment.

The most common side effects include:

- flu-like symptoms: fever, chills, headaches, and muscle aches
- frequent or painful urination
- urination at night
- traces of blood in your urine.

To help you manage these side effects, get plenty of bed rest, drink lots of liquids and take acetaminophen or ASA for any pain and fever. If you are concerned about your symptoms, contact your doctor.

This is not a complete list of side effects. Contact your doctor or pharmacist if you have any unexpected side effects while taking ImmuCyst®.

SERIOUS SIDE EFFECTS, HOW OFTEN THEY HAPPEN AND WHAT TO DO ABOUT THEM

If you experience the following symptoms, contact your doctor or get emergency help immediately:

- any sign of an **ALLERGIC REACTION**, which includes difficulty breathing, shortness of breath, wheezing, rash or hives and/or swelling of the face, or
- any sign of a **BCG INFECTION** which includes cough, high fever for more than 12 hours (greater than 39.5°C) or a fever (greater than 38.5°C) which lasts longer than two days.

If you notice the following symptoms, please see your doctor as soon as possible:

- yellow eyes or skin
- white or grey-coloured stools
- fever with chills, headache, muscle or joint pain that is not relieved by acetaminophen or ASA and lasts for more than 2 days
- severe pain or excessive urinating
- eye problems
- blood in urine

REPORTING SUSPECTED SIDE EFFECTS

To monitor drug safety, Health Canada collects information on serious and unexpected effects of drugs. If you suspect you have had a serious or unexpected reaction to this drug you may notify Health Canada by:
toll-free telephone: 866-234-2345
toll-free fax: 866-678-6789
By email: cadrmp@hc-sd.gc.ca

By regular mail:
National AR Centre
Marketed Health Products Safety and Effectiveness Information Division
Marketed Health Products Directorate
Tunney's Pasture, AL 0701C
Ottawa, ON K1A 0K9

NOTE: Before contacting Health Canada, you should contact your physician or pharmacist.

HOW TO STORE IT

ImmuCyst® should be stored at 2° to 8°C by your health-care provider.

MORE INFORMATION

This document plus the full product monograph, prepared for health professionals can be found at: www.sanofipasteur.ca or by contacting the sponsor,
Sanofi Pasteur Limited
1755 Steeles Avenue West
Toronto, Ontario, M2R 3T4

Phone: 1-888-621-1146 (no charge) or 416-667-2779.
Business hours: 8 a.m. to 5 p.m. Eastern Time
Monday to Friday.

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Last revised: January 2010

R11-0110 Canada

Cell line info

Cell Biology

ATCC® Number:	CRL-1593.2™	Order this Item	Price:	$272.00

Designations:	U-937
Depositors:	H Koren
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	monocyte
Source:	**Disease:** histiocytic lymphoma
Cellular Products:	lysozyme; beta-2-microglobulin (beta 2 microglobulin); tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-alpha, TNF alpha), after stimulation with phorbol myristic acid (PMA)
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The original U-937 cell line was established by Dr. K. Nilsson's laboratory in 1974 and he has requested the following: (1) In all papers reporting any use of this cell line or any derivatives thereof a direct reference should be made to Sundstrom and Nilsson (Int. J. Cancer 17: 565-577, 1976). (2) Any proposed commercial use of the cells should be negotiated with Professor Kenneth Nilsson, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden. (3) No distribution of any of the cells or sublines derived therefrom should be made to third parties; (4) The cells should be used for non-clinical, non-commercial research only.
Isolation:	**Isolation date:** 1974
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	complement (C3)
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 10,12 D16S539: 12 D5S818: 12 D7S820: 9,11 THO1: 6, 9.3 TPOX: 8,11 vWA: 14, 15
Age:	37 years
Gender:	male
Ethnicity:	Caucasian

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Product Information Sheet

Comments:	The U-937 cell line was derived by Sundstrom and Nilsson in 1974 from malignant cells obtained from the pleural effusion of a patient with histiocytic lymphoma. Studies since 1979 have shown that U-937 cells can be induced to terminal monocytic differentiation by supernatants from human mixed lymphocyte cultures, phorbol esters, vitamin D3, gamma interferon, tumor necrosis factor (TNF) and, retinoic acid. The cells are negative for immunoglobulin production and Epstein-Barr virus expression. The cells express the Fas antigen, and are sensitive to TNF and anti-Fas antibodies. In 1994, PCR and cytogenetic analyses showed that a number of stocks of U-937 were contaminated with the human myeloid leukemia cell line, K-562. In the earliest stocks available, the level of contamination was 0.6%. [40484] Distribution was discontinued in March 1994, except if required for patent purposes. Anyone who wishes to receive a sample of this original material should contact the Head of the ATCC Patent Depository. A stock of CRL-1593 found to be free of K-562 was propagated continuously for 8 weeks and tested weekly by PCR. Distribution and seed stocks give DNA profiles characteristic of U-937 only. Such preparations are now offered as authentic U-937 (ATCC CRL-1593.2) and are believed to be free of second subpopulations.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 to 2 X 10(5) viable cells/ml. **Interval:** Maintain cell density between 1 X 10(5) and 2 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 3 to 4 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020

References:

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Cell Biology

ATCC® Number:	**TIB-61™**	Order this Item	Price:	**$438.00**

Designations:	PU5-1.8 (PU5-1R)
Depositors:	P Ralph
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension (some adherent cells)
Organism:	*Mus musculus* (mouse)
Morphology:	
Source:	**Disease:** lymphoid tumor **Strain:** BALB/c
Cellular Products:	lysozyme; granulocyte colony stimulating activity (CSA) inducible by LPS
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Receptors:	complement (C3)
Comments:	PU5-1.8 cells phagocytose latex beads and zymosan. The cells are capable of antibody dependent lysis of both sheep erythrocytes and tumor cells. The line is sensitive to growth inhibition by LPS and PPD. Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** Dulbecco's modified Eagle's medium, 90%; horse serum, 10%
Subculturing:	**Medium Renewal:** Every 2 to 3 days Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 cells/ml and maintain between 1 X 10 exp5 and 1 X 10 exp6 cells/ml. Adherent cells can be recovered by scraping.
References:	1080: Ralph P, et al. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143: 1528-1533, 1976. PubMed: 1083890 1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031 1136: Ralph P, Nakoinz I. Direct toxic effects of immunopotentiators on monocytic myelomonocytic, and histiocytic or macrophage tumor cells in culture. Cancer Res. 37: 546-550, 1977. PubMed: 318922 1137: Ralph P, Nakoinz I. Lipopolysaccharides inhibit lymphosarcoma cells of bone marrow origin. Nature 249: 49-51, 1974. PubMed: 4208429

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Cell Biology

ATCC® Number:	**CRL-2055™**	Order this Item	Price:	**$365.00**
Designations:	NIT-1			
Depositors:	EH Leiter			
Biosafety Level:	2 [Cells contain polyomavirus DNA sequences]			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	Mus musculus, transgenic for SV40 large T antigen (mouse, transgenic for SV40 large T antigen)			
Morphology:	epithelial			
Source:	**Organ:** pancreas **Strain:** NOD/Lt **Tissue:** islet of Langerhans **Disease:** insulinoma **Cell Type:** beta cell;			
Cellular Products:	insulin			
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.			
Applications:	transfection host (Roche FuGENE® Transfection Reagents)			
Antigen Expression:	H-2 g7			
Age:	10 weeks			
Gender:	female			

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Comments:	The NIT-1 cell line was derived from NOD/Lt mice. These mice are transgenic for the SV40 large T antigen under the control of a rat insulin promoter, and spontaneously develop beta adenomas. At passage 18, most cells stained positively for insulin, less than 5% were positive for glucagon and none were positive for somatostatin or pancreatic polypeptide. Insulin secretion is responsive to glucose concentration in the medium. There is low constitutive expression of MHC class I, class II and ICAM-1 mRNA, but expression of both is markedly increased by treatment with interferon gamma. Stimulation of class I mRNA is accompanied by increased class I antigen expression and induction of an occult class I product expressing the H-2.39 specificity. MHC class II antigen is not induced despite the induction of the mRNA. NIT-1 cells show ultrastructural features of differentiated mouse beta cells (well developed rough endoplasmic reticulum, extensive golgi apparatus and beta granules). The cells shed a mature ecotropic type C retrovirus. The retrovirus is capable of infecting other Fv-1 n mouse cell lines, so care should be taken to avoid cross infection. NOTE: NIT-1 cells will not form a confluent monolayer; however, they will form nice colonies of monolayered cells in a fairly dense array. When the NIT-1 colonies begin to ball up slightly and show many round cells on top of the monolayers as well as floating in the media, it is time to passage them.
Propagation:	**ATCC complete growth medium:** Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 90%; heat-inactivated dialyzed fetal bovine serum, 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended **Medium Renewal:** 1 to 2 times per week
Subculturing:	Subcultures are prepared using a cell dissociation buffer (an enzyme free Hanks' based solution; Catalog number: 13150-016 available from GIBCO). Remove the medium from the culture flask, add 2 ml of cell dissociation buffer per 25 sq. cm flask (5 ml per 75 sq. cm. flask and gently rock the flask at room temperature for 1 to 2 minutes to bathe the cells in the buffer. Aspirate the solution and discard. Allow the flask to sit at room temperature for 3 to 4 additional minutes (total time from initial addition of cell dissociation buffer is approximately 5 minutes). Firmly tap the flask against the palm of the hand to dislodge cells. Add 5 ml of fresh medium per 25 sq. cm. flask (10 ml per 75 sq. cm. flask) and triturate up and down directing the stream along the bottom of the flask to dislodge the cells and break up some of the clumps. Add fresh medium, aspirate and dispense into new flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
References:	22641: Hamaguchi K, et al. NIT-1, a pancreatic beta-cell line established from a transgenic NOD/Lt mouse. Diabetes 40: 842-849, 1991. PubMed: 1647994

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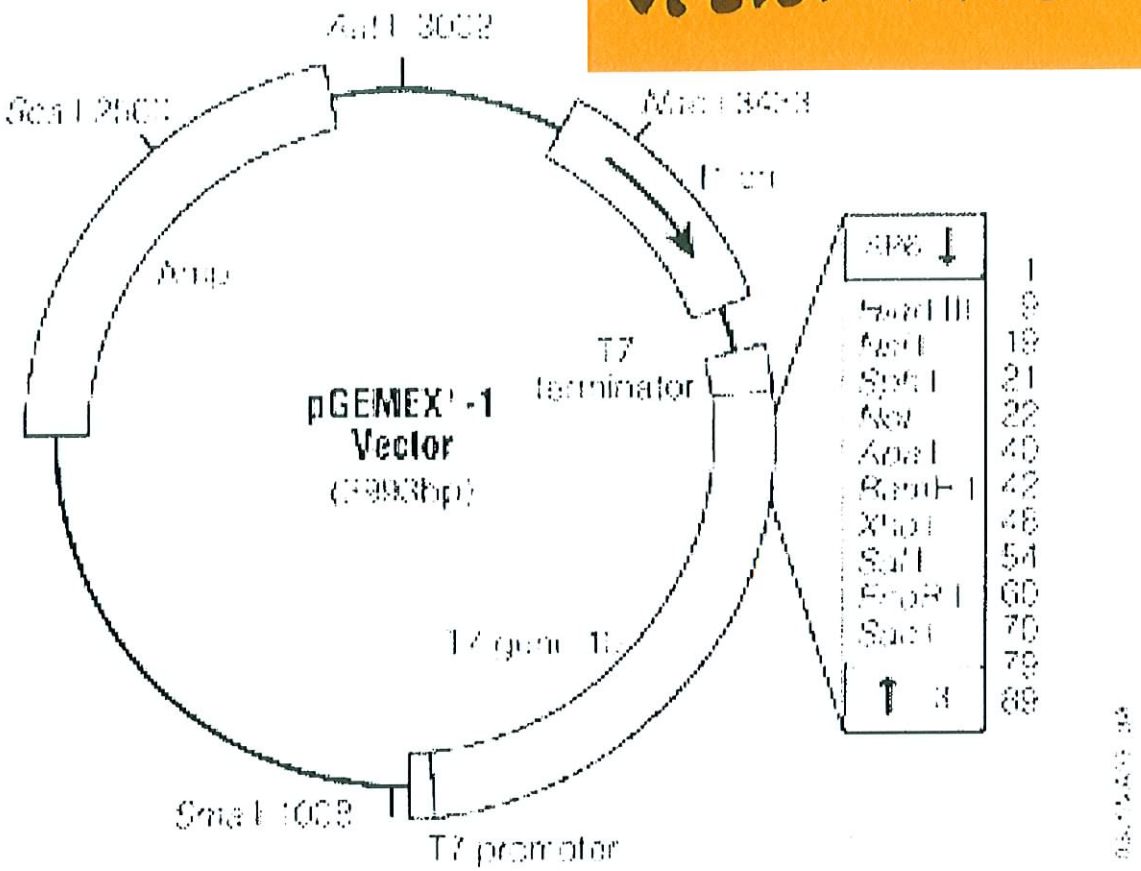
pGEMEX-1 vector and Insert DNA for Cloning

Vector Info



Toxin Info

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.1
Revision Date 06/18/2009
Print Date 08/04/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Staphylococcal enterotoxin B, from *Staphylococcus aureus*

Product Number : S4881
Brand : Sigma

Company : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Telephone : +19058299500
Fax : +19058299292
Emergency Phone # : 1-800-424-9300

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Enterotoxin B, Staphylococcal

CAS-No.	EC-No.	Index-No.	Concentration
Staphylococcal enterotoxin B Staphylococcus aureus			
11100-45-1	-	-	-

3. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Small intestine.

WHMIS Classification

D1B Toxic Material Causing Immediate and Serious Toxic Effects — Toxic

HMIS Classification

Health Hazard: 0
Chronic Health Hazard: *
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation.

Environmental precautions
Do not let product enter drains.

Methods for cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
For prolonged or repeated contact use protective gloves.

Eye protection
Safety glasses with side-shields conforming to EN166

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

no data available

Irritation and corrosion

no data available

Sensitisation

Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

Chronic exposure

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.
Target Organs	Small intestine.,

Additional Information
RTECS: XW5807700

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3462 Class: 6.1 Packing group: III
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Staphylococcal enterotoxin B Staphylococcus aureus)
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 3462 Class: 6.1 Packing group: III EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Staphylococcal enterotoxin B Staphylococcus aureus)
Marine pollutant: No

IATA
UN-Number: 3462 Class: 6.1 Packing group: III
Proper shipping name: Toxins, extracted from living sources, solid n.o.s. (Staphylococcal enterotoxin B Staphylococcus aureus)

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Staphylococcal enterotoxin B Staphylococcus aureus	11100-45-1

WHMIS Classification

D1B	Toxic Material Causing Immediate and Serious Toxic Effects	Toxic

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Material Safety Data Sheet

Version 4.0
Revision Date 03/13/2010
Print Date 08/04/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Pertussis toxin, from *Bordetella pertussis*
Product Number	:	P7208
Brand	:	Sigma
Company	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA
Telephone	:	+19058299500
Fax	:	+19058299292
Emergency Phone #	:	1-800-424-9300

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Pancreas.

WHMIS Classification

D1A D1B	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by inhalation Toxic by ingestion Toxic by skin absorption

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H302	Harmful if swallowed.
H311	Toxic in contact with skin.
H330	Fatal if inhaled.

Precautionary statement(s)

P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing.
P284	Wear respiratory protection.
P310	Immediately call a POISON CENTER or doctor/physician.

HMIS Classification

Health hazard:	4
Chronic Health Hazard:	*
Flammability:	0
Physical hazards:	0

Potential Health Effects

Inhalation	May be fatal if inhaled. May cause respiratory tract irritation.
Skin	Toxic if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	Toxic if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Islet Activating Protein
IAP
Pertussigen
Histamine-sensitizing factor

CAS-No.	EC-No.	Index-No.	Concentration
Pertussis toxin from Bordetella pertussis			
70323-44-3	-	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Face shield and safety glasses

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder, lyophilized

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity
LD50 Intravenous - rat - 0.114 mg/kg
Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste):Eye:Lacrimation. Behavioral:Change in motor activity (specific assay). Nutritional and Gross Metabolic:Weight loss or decreased weight gain.

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure (GHS)
no data available

Specific target organ toxicity - repeated exposure (GHS)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be fatal if inhaled. May cause respiratory tract irritation.
Ingestion	Toxic if swallowed.
Skin	Toxic if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
Potentially neurotoxic.

Additional Information
RTECS: XW5883750

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 3462 Class: 6.1 Packing group: I EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: No

IATA
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Pertussis toxin from Bordetella pertussis)

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Pertussis toxin from Bordetella pertussis	70323-44-3

WHMIS Classification

D1A D1B	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by inhalation Toxic by ingestion Toxic by skin absorption

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Laboratory Exposures to Staphylococcal Enterotoxin B

Janice M. Rusnak,* Mark Kortepeter,* Robert Ulrich,* Mark Poli,* and Ellen Boudreau*

Staphylococcal enterotoxins are 23- to 29-kDa polypeptides in the bacterial superantigen protein family. Clinical symptoms from intoxication with staphylococcal enterotoxins vary by exposure route. Ingestion results in gastrointestinal symptoms, and inhalation results in fever as well as pulmonary and gastrointestinal symptoms. Review of occupational exposures at the U.S. Army Medical Research Institute of Infectious Diseases from 1989 to 2002 showed that three laboratory workers had symptoms after ocular exposure to staphylococcal enterotoxin B (SEB). Conjunctivitis with localized cutaneous swelling occurred in three persons within 1 to 6 hours after exposure to SEB; two of these persons also had gastrointestinal symptoms, which suggests that such symptoms occurred as a result of exposure by an indirect cutaneous or ocular route. Ocular exposures from SEB resulting in conjunctivitis and localized swelling have not previously been reported. Symptoms from these patients and review of clinical symptoms of 16 laboratory-acquired inhalational SEB intoxications may help healthcare workers evaluate and identify SEB exposures in laboratory personnel at risk.

Staphylococcal enterotoxins are 23- to 29-kDa polypeptides in the bacterial superantigen protein family that act by cross-linking HLA-DR or DQ molecules and T-cell receptors. This cross-linking results in potentially pathologic levels of proinflammatory cytokines, such as tumor necrosis factor α, interleukin 2, and interferon-γ (1,2). Therefore, symptoms of mild exposure are anticipated to resemble T-cell–mediated recall responses, similar to a Mantoux skin test.

Staphylococcal enterotoxin B (SEB) is one of at least 15 antigenically distinct enterotoxin proteins (3,4). Clinical symptoms depend on the route of exposure. After inhalation of SEB, clinical features include fever, respiratory complaints (cough, dyspnea, and retrosternal discomfort or chest pain), and gastrointestinal symptoms; severe intoxication results in pulmonary edema, adult respiratory distress syndrome, shock, and death (5,6). Ingesting SEB may cause food poisoning within 1 to 6 hours of exposure, manifested as acute salivation, nausea, and vomiting, followed by abdominal cramps and diarrhea (7,8). As ingesting SEB does not typically result in pulmonary symptoms, gastrointestinal symptoms observed from inhalational intoxication are postulated to result from secondary oral ingestion of SEB concomitant with the inhalational exposure.

One laboratory incident that resulted in nine cases of inhalational intoxication to SEB and several other outbreaks of food poisoning from ingesting staphylococcal enterotoxins have been reported in the literature (5). Symptoms occurring after ocular exposure and localized cutaneous swelling or conjunctivitis from staphylococcal enterotoxins have not been reported. We report three cases of purulent conjunctivitis with localized facial swelling that occurred after ocular exposure to SEB in the laboratory. Two of the three patients also complained of gastrointestinal symptoms. The symptoms in these three mucocutaneous-acquired cases, and summary of symptoms from 16 laboratory-acquired inhalational intoxications with SEB, may help define the clinical spectrum that might be expected after SEB exposures. The full spectrum of clinical signs and symptoms of intoxication with SEB is important to healthcare workers evaluating persons with potential exposures to these agents, including in the context of bioterrorism. This discussion is also relevant to military practitioners, since SEB has been previously developed as an incapacitating biowarfare agent.

Methods

During a review of occupational exposures evaluated in the Special Immunizations Clinic at the U.S. Army Medical Research Institute of Infectious Diseases from 1989 to 2002, clinical evaluations of three laboratory workers with symptoms of conjunctivitis and localized swelling after exposure to SEB were identified. Patient records and occupational exposure summaries were reviewed. Additionally, clinical histories of 16 persons with symptoms after inhalational intoxication with SEB,

*United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA

obtained from both that research facility's medical records and occupational exposure reports, were reviewed to summarize the spectrum of symptoms resulting from inhalational exposure to SEB.

Results

Patient 1

While injecting SEB into the endotracheal tube of a rabbit, a 22-year-old male laboratory worker sprayed approximately 150 μg of SEB onto his right glove. Sometime later, he recalled scratching his nose and the area adjacent to his right eye. Three hours after the incident, he noted irritation, pruritus, and a yellow discharge from his right eye. Nine to 12 hours after the incident, he had onset of gastrointestinal symptoms (nausea, abdominal cramps, and loose stools [approximately eight nonbloody loose stools over the next 8 hours]), nasal congestion, postnasal drip, and a self-reported fever. The following morning (approximately 18 h after the incident), he awoke with profound swelling of the right lower eyelid and passed three more loose stools. He did not have headache, chills, vomiting, cough, dyspnea, chest discomfort, or myalgias.

Physical examination was remarkable for diffuse hyperemia of the eye, mildly edematous conjunctiva inferiorly, and edema of the lower lid. The patient was given loperamide for control of diarrhea and sulfacetamide ophthalmic ointment to the right eye four times daily. Gastrointestinal symptoms resolved within 2 days, and the ocular symptoms, nasal congestion, postnasal drip, and febrile symptoms resolved within 4 days. The laboratory worker discontinued loperamide at day 2 and sulfacetamide ointment at day 4.

The amount of SEB in the spray was estimated to be <150 μg. However, the amount of SEB exposure to the right eye was even less, since only a portion of the solution was sprayed onto his hand and rubbed into his eye. The worker was wearing a Tyvek suit (DuPont, Wilmington, DE) and gloves at the time of the exposure but no goggles or respirator. As a consequence, safety measures were modified to recommend only Leur-Lock syringes (Baxter International Inc., Deerfield, IL) and to require eye protection and surgical masks while working with the toxin.

Patient 2

During the reconstitution of SEB within a biosafety cabinet, a 20-year-old laboratory worker injected the contents of a syringe containing 500 μg of SEB into a sealed vial and was exposed when the solution in the vial came under pressure. Approximately 100 μL of SEB in solution foamed from the sealed vial. A small portion of the solution came into contact with the fourth finger on her left hand. She was not wearing gloves. She immediately washed the site with soap and water for 15 seconds and rinsed the soap from her hands. Before she dried her hands, she unconsciously rubbed her left eye with her left hand.

Within 6 to 9 hours of exposure, she had onset of a thick mucous discharge from her left eye, a swollen eye lid, nausea, and loose stools. She had no fever, headache, cough, dyspnea, chest discomfort, vomiting, or myalgias. She was seen at a local emergency room that evening, and was given gentamicin eye drops with a presumed diagnosis of "pink eye" and phenergan for nausea. She was told to remove her contact lenses. The following morning, she was seen in the Special Immunizations Clinic for evaluation for a potential occupational exposure, after reporting to her supervisor that her symptoms might be related to contact with SEB the previous day. Physical examination showed swelling of the left eyelid and discharge from the eye. The discharge was initially described as "long threads" and was subsequently noted to have a yellow color and tear-like appearance. Her symptoms of nausea and diarrhea continued; symptoms exacerbated with food intake. The gastrointestinal symptoms resolved in 3 days, and the ocular symptoms in 5 days.

The estimated syringe loss was 500 μg, but the amount of exposure was estimated to be ≤50 μg, since only a small portion of the solution was in contact with her hand. Because the toxin is extremely water soluble, the immediate washing of the hands should have effectively removed a large amount of the toxin.

Patient 3

One hour after cleaning a dime-size amount of liquid, likely to have been SEB, found outside a biosafety cabinet, a 23-year-old laboratory technician noted bilateral eye irritation, conjunctival erythema, and an excessive yellow ocular discharge that continued throughout the day. She awoke the next morning (day 2) with facial swelling, persistent ocular symptoms, and a subconjunctival hemorrhage (possibly resulting from SEB transfer from hand to eye). She medicated herself with diphenhydramine and brompheniramine and noted improvement in her symptoms the following day (day 3).

On the morning of day 3, she visited the Special Immunizations Clinic for evaluation. At that time, the facial swelling had resolved, and the ocular symptoms had nearly resolved. She had no fever, headache, cough, dyspnea, chest discomfort, nausea, vomiting, diarrhea, or myalgias. Physical examination was remarkable for bilateral conjunctival injection and a 5-mm x 2-mm subconjunctival hemorrhage at the inferolateral margin of the right iris. Complete blood count and erythrocyte sedimentation rate were within normal limits. She was seen by her private ophthalmologist later that day, who recommended no specific treatment. Her symptoms resolved on day 4.

Subsequently, this patient had mild ocular erythema and irritation (no facial swelling or conjunctivitis) while in the laboratory where SEB studies were ongoing. These symptoms resolved immediately after she left the room, which suggests hypersensitivity. She was advised to avoid entering the laboratory when SEB was being used or consider prophylactic use of antihistamines to control symptoms.

Inhalational Cases

Three historical events that occurred during the now disbanded U.S. offensive biologic warfare program resulted in inhalational exposures to SEB and subsequent intoxication. The spectrum of symptoms occurring in the three events is summarized in the Table.

In early 1963, two persons were exposed to SEB as a result of a ruptured hose that contained a crude filtrate of SEB under moderate pressure. Acute asthmatic bronchitis developed in one of these persons within a few hours of exposure. Fever, headache, myalgias, nonproductive cough, dyspnea, nausea, vomiting, and diarrhea developed in both persons, with maximal symptoms at 12 hours and resolution of symptoms by day 3.

In June 1963, five of seven persons became ill within 24 hours of exposure to a highly purified SEB aerosol after a dose-titration experiment in monkeys using a Henderson apparatus for administration of the aerosol; four of the persons required hospitalization (9). The source was suspected to be residual SEB from fur on the monkeys' heads, which had not been wiped after the exposure to SEB. Fever, cough, sternal tightness, anorexia, nausea, and vomiting were prominent features of intoxication; these signs and symptoms started within a few hours to as late as 24 hours after exposure.

The third event occurred in August 1964, when a leak in tubes carrying aerosolized SEB to test monkeys resulted in exposure of 15 persons. Ten persons became symptomatic, and 9 of them were hospitalized (5).

Onset of symptoms from inhalational intoxication was within 1–1/2 hours to 24 hours of exposure (most within

Table. Signs and symptoms of inhalational intoxication to staphylococcal enterotoxin B

Signs and symptoms	Event 1[a] (1963) N = 2	Event 2[b] (1963) N = 4	Event 3 (1964) N = 10	Total (%)
Generalized				
Fever	2	4	9	15/16 (93.7)
Chills	2	2	9	13/16 (81.3)
Headache	2	2	9	13/16 (81.3)
Myalgia	2	1	8	11/16 (68.7)
Fatigue	ND[c]	2	8	10/14 (71.4)
Malaise	ND	2	7	9/14 (64.3)
Lower respiratory				
Cough	2	3	10	15/16 (93.7)
Dyspnea	2	2	4	8/16 (50.0)
Retrosternal or chest pain	ND	3	5	8/14 (57.1)
Wheezing	1	0	1	2/16 (12.5)
Gastrointestinal				
Nausea	2	4	6	12/16 (75.0)
Vomiting	2	3	4	9/16 (56.3)
Anorexia	2	2	5	9/16 (56.3)
Abdominal cramps	ND	1	2	3/14 (21.4)
Diarrhea[d]	2	0	0	2/16 (12.5)
Gas	ND	0	1	1/14 (7.1)
Hepatitis	0	0	1	1/14 (7.1)
Upper respiratory				
Pharyngeal injection	ND	2	3	5/14 (35.7)
Rhinorrhea, postnasal drip, or sinus congestion	ND	2	2	4/14 (28.6)
Sore throat	ND	1	2	3/14 (21.4)
Otitis	ND	1	1	2/14 (14.3)
Hoarseness	ND	0	1	1/14 (7.1)
Other				
Conjunctival injection	ND	2	2	4/14 (28.6)
Burning eyes	ND	0	1	1/14 (7.1)
Flushed face	ND	1	0	1/14 (7.1)

[a]Only occupational summary reports reviewed (medical records not available).
[b]No records available on the one nonhospitalized symptomatic person.
[c]ND, no data.
[d]Loose stools noted in one person in the second and the third events.

12 hours of exposure), and the duration of symptoms was generally 3–5 days. In addition to the previously reported and commonly observed symptoms of fever, headache, myalgias, pulmonary symptoms, and gastrointestinal symptoms, fatigue and malaise were observed in most persons (Table). While diarrhea was reported in a few cases, it was not a prominent finding with SEB intoxication by inhalation. Conjunctival injection, previously reported in the literature, was noted only in four persons.

Newly reported symptoms include upper respiratory symptoms (e.g., sore throat, profuse postnasal drip, sinus congestion, rhinorrhea, coryza, and hoarseness). Pharyngeal injection (five persons) and injected tympanic membranes (two persons) were observed; neither had been previously reported in the literature.

Discussion

Outside the context of food poisoning, few physicians would be expected to have experience evaluating persons with SEB intoxication. However, an increase in laboratory exposures and intoxications with staphylococcal enterotoxins can be expected as additional institutions begin work with them as a result of increased funding for biodefense research. Symptoms of conjunctivitis with periocular or facial swelling, acquired by ocular or cutaneous exposure routes, have not been previously reported in the literature. A historical occupational health Department of Defense report suggests that conjunctivitis and upper respiratory tract symptoms resulting from exposures to staphylococcal enterotoxins were recognized during the time of the U.S. offensive biological warfare program from 1945 to 1969 (10). Therefore, documenting the full clinical spectrum of intoxications with staphylococcal enterotoxins is necessary to educate healthcare workers and safety officers to enable them to identify workers at risk and prevent exposures to staphylococcal enterotoxins.

Clinical symptoms from SEB may vary and are dependent on the dosage and route of exposure (5). While inhaling SEB may result in fever, pulmonary, and gastrointestinal symptoms; ingestion of staphylococcal enterotoxins generally results mainly with gastrointestinal symptoms. The gastrointestinal symptoms noted in two persons with ocular or percutaneous exposures (or both) suggest that gastrointestinal symptoms from SEB may occur by a nonoral route, although transmission of SEB to the gastrointestinal tract via the lacrimal duct cannot be entirely excluded. Also, recurring symptoms of ocular irritation and erythema when in the presence of SEB, and immediate resolution of symptoms when no longer in an SEB area, suggests a possible hypersensitivity to SEB.

The pathophysiology of symptoms from staphylococcal enterotoxins, however, is not fully understood. Staphylococcal enterotoxins are superantigens that act by cross-linking HLA-DR or DQ molecules and T-cell receptors, resulting in high levels of inflammatory cytokines such as interleukin 2, interferon-γ, and tumor necrosis factor (1). Staphylococcal enterotoxins resist inactivation by gastrointestinal proteases; oral dosages as low as 5–20 μg induce emesis in nonhuman primates (4,8). However, nongastrointestinal routes such as intravenous administration of SEB (higher dosages of 20 to 500 μg) also induced emesis in nonhuman primates.

High levels of cytokines alone may cause symptoms similar to SEB intoxication. Cancer patients, given high doses intravenously of the cytokine interleukin-2, have symptoms of fever, malaise, nausea, vomiting, and diarrhea, similar to SE food poisoning (7). Also, intravenous OKT3, a monoclonal antibody used as an immunosuppressant in transplant patients (it binds to T lymphocytes, resulting in early activation of T cells, cytokine release, and subsequent blocking of T-cell functions), has a side effect profile similar to that of SEB—high fever, gastrointestinal symptoms, arthralgias, and pulmonary symptoms (11).

The mechanism of emesis also has been postulated to be related to the stimulation of mast cells and the subsequent release of cysteinyl leukotrienes and histamine (12). L4 171883, a selective inhibitor of LTD4/LTEF receptors, completely eliminated the emetic response and immediate type skin reactions (skin reactions associated with degranulation of cutaneous mast cells) to SEB. Inhibition of prostaglandins by indomethicin or pretreatment with a dual lipoxygenase and cyclo-oxygenase inhibitor (BW 755C) did not prevent emesis or immediate type skin reactions. After degranulation of mast cells, impulses are sent through the vagus and sympathetic nerves to the medullary center, which results in emesis. Severing of the vagus and sympathetic nerves inhibits the emesis response (13).

The mechanism of diarrhea is even less well understood, although it is not by means of activation of adenylate cyclase (5). Histopathologic findings with staphylococcal food poisoning are minimal: they mainly show polymorphonuclear cell infiltrates in the epithelium and lamina propria of the stomach and proximal small intestine (7,8).

SEB intoxication is diagnosed by clinical symptoms and a history of potential exposure to SEB. Definitive diagnosis of inhalational exposure can be made by nasal swabs and induced respiratory secretions for toxin assays, blood and urine for immunoassay, and acute- and convalescent-phase serum, but these tests are not readily available and not reliable for low-dose exposures. While inhalational intoxication with SEB is generally associated with leukocytosis and a mildly elevated erythrocyte sedimentation rate, these findings are nonspecific. Chest x-ray findings of increased interstitial markings, atelectesis, overt pulmonary edema, or acute respiratory distress

syndrome are also nonspecific and only present in inhalational intoxications of SEB (5). Potential changes in serum antibody titers, although relevant, have not been examined.

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 (14). 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 (14). 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.

During the offensive biologic warfare program, a contractor report addressing the efficacy of biosafety cabinets noted toxic reactions in persons performing SEB purification studies on open laboratory benches (10). The following symptoms were noted in six persons: conjunctivitis, nondescript chemical irritation of one eye, general skin reaction, severe facial skin reaction, dermatitis, and cold symptoms. Additionally, symptoms mainly of conjunctivitis and acute pharyngitis, but also including vomiting and diarrhea in two cases, were observed in 23 persons wearing surgical masks or face shields while working with SEB. Persons working with SEB within a biosafety cabinet had no symptoms.

As exposure to low dosages of SEB can produce symptoms, these recently reported symptoms have importance both to safety officers and healthcare workers evaluating laboratory workers at risk with potential exposures to staphylococcal enterotoxins. SEB intoxication can mimic an infectious process. The initial diagnosis of the first person who sought medical evaluation in the June 1963 incident was pneumococcal pneumonia; symptoms included acute onset of fever, chills, productive cough, chest pain, and dyspnea. The patient was started on penicillin, which was discontinued after his co-workers exhibited similar symptoms, a finding that supported the diagnosis of SEB intoxication. Even though medical providers had knowledge of SEB exposure in a subsequently hospitalized patient involved in the June 1963 incident, the initial symptoms of this patient still raised concern about a possible infectious cause. That patient was noted to have a flushed face, mild hyperemia of the pharynx, a prominent postnasal drip, a purulent-appearing otitis media and externa without symptoms of ear pain, pulmonary symptoms (productive cough and chest discomfort), and a leukocytosis of 19,500 cells/mm^3. Their differential diagnosis included otitis externa and media, pneumonia, or SEB intoxication. Otic examination was within normal limits 24 hours later, which suggested SEB as the possible cause of the localized swelling. An infectious cause was also considered as the initial primary diagnosis in the initial two patients with conjunctivitis in this series as the cause of the conjunctivitis, gastrointestinal symptoms, or both, with both persons receiving topical ophthalmic antimicrobial agents for conjunctivitis. Healthcare workers evaluating persons who work with SEB need to be aware of the full spectrum of toxicity symptoms associated with SEB to avoid misdiagnosis resulting in unnecessary treatment, to identify breaches in laboratory technique, and to educate persons at risk of the importance of personal protective measures in preventing SEB exposure and intoxication. These cases emphasize that personal protective measures such as biosafety cabinets, gloves, and eye protection are paramount when working with SEB.

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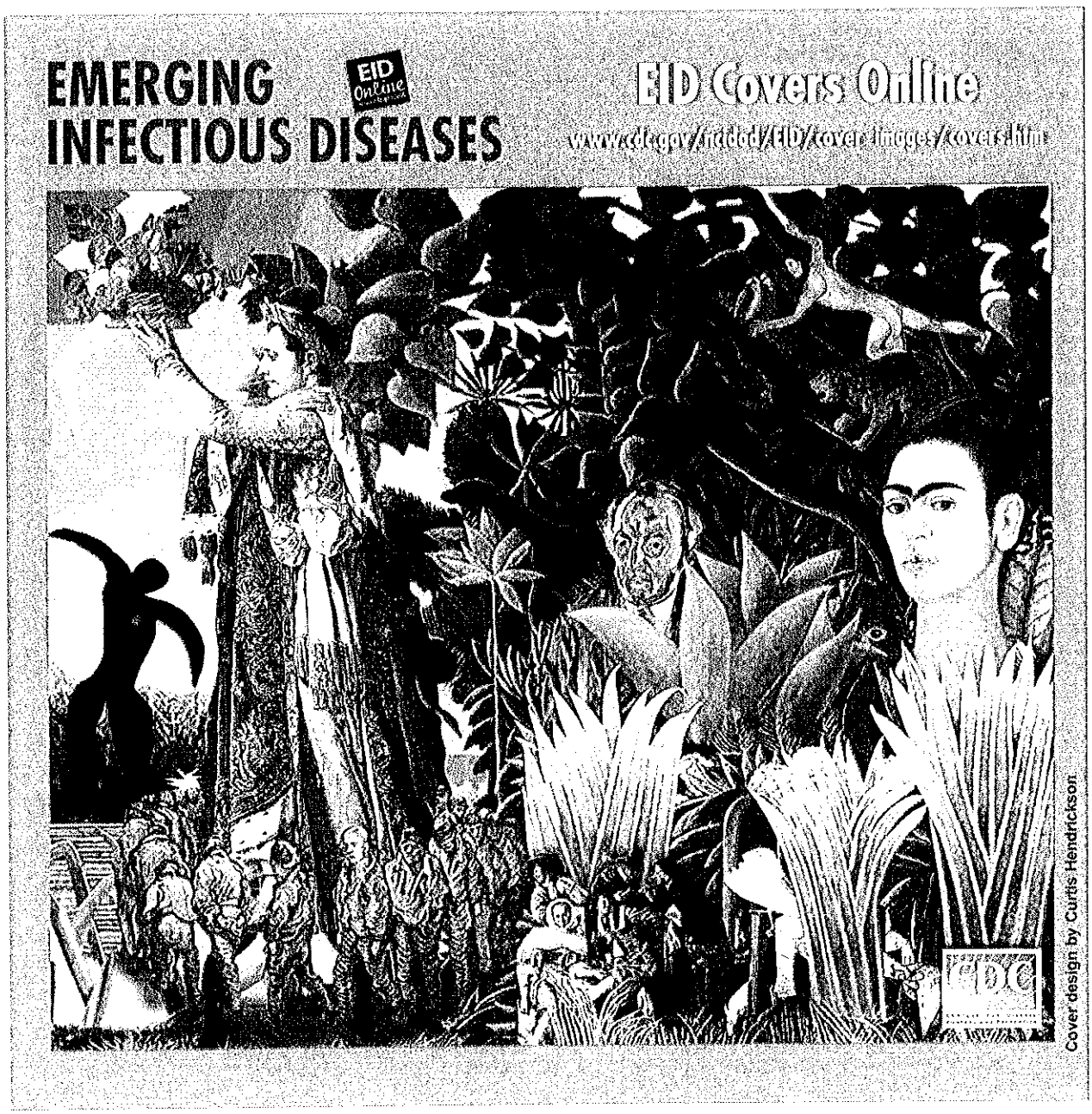

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Staphyloccoccal Enterotoxin B
Proposed Use Dose:	0.006 µg
Proposed Storage Dose:	5000 µg
LD $_{50}$ (species):	0.02 µg

Calculation:

0.02 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 1

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = *0.1*

Comments/Recommendations:
Storage is over limit, therefore should be stored in at least 2 different locations?

Toxin Calculations

Western

TOXIN USE RISK ASSESSMENT

TOXIN: Pertussis toxin

PROPOSED USE (DOSE): 4 µg (use) or 50 µg (storage)

LD_{50} (species): 0.114 mg/kg (rat)
or 114 µg/kg (rat)

CALCULATION:

114 ug/kg X 70 kg/person = 7980 ug per person

Divide by safety factor(s) of 10 (as applicable): 798 ug per person

COMMENTS/RECOMMENDATION:

798 µg/person >> 4 µg (use)
∴ OK

> 50 µg (storage)

∴ OK

- Recommend vaccinations be up-to-date? ...